THE CHEMOSEROTHERAPY OF EXPERIMENTAL PNEUMOCOCCAL INFECTION.

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In a former communication (1) we reported that the action of ethylhydrocuprein (a derivative of hydroquinine introduced by Morgenroth and Levy (2)) was well marked *in vitro* and *in vivo* on type strains of the four groups of pneumococci. Since we possess in certain types of antipneumococcus serum the means of conferring on experimental animals considerable specific protection against very many multiples of the minimal lethal dose of a virulent pneumococcus culture, it seemed to us of interest to study the combined effect of chemo- and serotherapy *in vivo,* and to endeavor to get some numerical values representing the results.

Neufeld (3) showed that strains of pneumococci differed among themselves in respect to their immunity reactions (protective antibodies).

The work of Cole (4) and of Dochez and Gillespie (5) has resulted in a serological classification of the pneumococci. The pneumococci can be divided into at least four groups; an immune serum produced against any member of Group I has a specific agglutinative action upon, and a specific protective action against, any member of Group I, but has no such effects on any member of the other three groups. In like manner, an immune serum against any member of Group II behaves similarly with respect to any member of Group II, but has no effect on any member of Groups I, III, or IV. To Group III belong all the microorganisms of the *Pneumococcus mucosus* type. To Group IV belong all those strains of pneumococci which do not fall into the other three groups; an immune serum produced against any member of this group has a specific agglutinative action upon, and a specific protective action against, the strain used for its production, but has no such effects on any other member of this group, or of the other groups.

Neufeld and Engwer (6) and Engwer (7) have studied the effect of combining the serum and chemotherapy in guinea pigs infected with pneumococcal pleural exudates; these observers saw an increased effect by the combination. Their protocols do not show, however, to what group either the pneumococcus or antiserum belonged; it is not clear whether or not the antiserum used was

produced against a member of the group to which the infecting pneumococcus belonged. Boehncke (8), too, saw a markedly increased effect in mice by the combination of serum and drug therapy over and above the effect of either of these two separately; in the protocols of his experiments, also, we have little information as to the group to which the pneumococcus or antiserum belonged.

We deemed it of importance to study the results of combined chemotherapy and serum therapy in the light of the serological classification of the pneumococci mentioned above; and, since the results of serum therapy in man have not been at all as satisfactory in the case of lobar pneumonia due to pneumococci belonging to Group II as when the disease is caused by a strain of Group I, we have given the greater part of our attention, in this respect, to infection with pneumococci belonging to Group II. Further, the threshold value of our immune horse serum to Group I is more than ten times greater than the corresponding serum to Group II. Hence a satisfactory result with an antiserum to Group II should mean an even better result with an antiserum to Group I, in the case of infection with a strain of the homologous group.

Since toxic symptoms have been several times noticed in human patients and in experimental animals treated with ethylhydrocuprein, we have endeavored to reduce to a minimum the amount of ethylhydrocuprein given to the animals in the present study. The dose of ethylhydrocuprein (optochin base) recommended by Morgenroth (9) in experimental pneumococcal infection in the mouse is 0.5 cc. of a 2 per cent solution in olive oil given under the skin of the back immediately following the infection, and repeated on the next day; this is followed on the third day, and on the fourth day if desirable, by a similar injection of 0.4 cc. of the same solution. The dosage used in the present study is considerably below this. The experimental animals used were mice.

EXPERIMENTAL.

The strain of pneumococcus of Group II used in the present study was a stock strain and was maintained in a condition of maximal virulence throughout the experiments; that is to say, 0.000,001 cc. of a twenty hour broth culture regularly killed mice within forty-eight hours. Several controls of virulence were done in the case of each

experiment. A twenty hour broth culture of the pneumococcus was used in every case. In the designation of the culture, the Roman numeral represents the group to which the pneumococcus belongs, the Arabic figure the number of animal passages which the strain had had, and the exponent the number of cultivations on artificial media since the last animal passage.

Mice of 18 grams' weight and upwards were used; the dosage of the drug was calculated in every case according to the weight of the animal. An autopsy was performed on every mouse that succumbed, unless otherwise stated in the protocols (owing to the body having been eaten by the survivors).

A constant amount of immune horse serum, 0.2 cc., was used in the experiments; it was mixed with the infecting dose of culture, and the mixture, making a total volume of 1 cc., was given intraperitoneally.

The ethylhydrocuprein (optochin base) was administered in the form of a 2 per cent solution in olive oil, given under the skin of the back. This treatment was given, in the first instance, immediately

Experiment 1. Titer of Antipneumococcus Serum II.—(Table I.) This serum protected regularly against 0.001 cc. of a 20 hour broth culture of any strain of pneumococcus belonging to Group II.

TABLE I.

Culture II 42^5; Virulence Maximal.

Amount of culture.	Result.	Amount of culture.	Result.	Amount of culture.	Result.
cc.		*cc.*		*cc.*	
0.001	S	0.02	■ 48 hrs.	0.08	■ 24 hrs.
"	"	"	■ 60 "	"	■ " "
"	"	0.04	S	"	■ " "
"	"	"	"	"	■ " "
"	"	"	"	"	■ " "
"	"	"	■ 48 "	"	■ " "
"	"	"	■ " "	"	■ " "
"	"	0.06	■ 24 "	"	■ " "
0.01	"	"	■ 48 "	"	■ 48 "
"	"	"	■ " "	"	S
"	"	"	■ " "	0.1	■ 24 "
"	"	"	■ " "	"	■ " "
"	■ 72 hrs.	"	■ " "	"	■ " "
"	■ " "	"	■ 72 "	"	■ 48 "
0.02	S	"	■ " "	"	■ " "
"	"	"	■ " "	"	■ " "
"	■ 30 "	"	■ " "		

after the infection; if a second dose was given it was given after an interval of 24 hours.

In the protocols the letter S stands for survival of the corresponding animal; a black square (■) means death of the animal from pneumococcal septicemia, capsulated diplococci having been found in its heart's blood by His's capsule stain, or, if this examination proved negative, Gram-positive diplococci having been recovered by culture (abundant inoculation on defibrinated rabbit blood agar) from the heart's blood at autopsy. An oblique cross (X) means death of the animal, the bacteriological examination of the heart's blood (and peritoneal cavity, if necessary) by smear and culture being negative; in this case the death of the animal may have been due to fortuitous influences, the toxicity of the drug, traumatism inflicted in the experiment, etc. When an autopsy was not possible on an animal, its death is indicated by the sign +.

Comment on Experiment 1.—From the protocol it is seen that, while the intraperitoneal injection of 0.2 cc. of antipneumococcus serum II gives to mice a sure protection against 0.001 cc. of a virulent culture (Strain II 42^5), this amount of serum only gives a very doubtful protection against 0.01, 0.02, and 0.04 cc. of culture, and none at all against 0.06 cc. or more.

Experiment 2.—Effect of a single dose of 0.45 cc. and of a single dose of 0.5 cc., respectively, of a 2 per cent solution of ethylhydrocuprein (optochin base) in olive oil per 20 gm. of mouse on pneumococcal infection. (Table II, Strain II 42^6.)

TABLE II.

Mouse No.	Amount of culture.	Normal horse serum.	Ethylhydrocuprein 2 per cent solution.	Result.	
				2d day.	3d day.
	cc.	*cc.*	*cc.*		
1	0.08	—	0.45	Sick	■
2	"	—	"	"	■
3	"	—	"	"	■
4	"	—	"	■	
5	"	0.2	"	Sick	■
6	"	"	"	"	■
7	"	"	"	"	■
8	"	"	"	"	■
9	0.001	—	0.5		■
10	"	—	"	"	■
11	"	—	"	Well	■
12	0.0001	—	"	Sick	■
13	"	—	"	■	
14	"	—	"	Well	■

Comment on Experiment 2.—A single treatment with either 0.45 cc. or 0.5 cc. of the 2 per cent solution of the drug is without effect on the lethal issue due to pneumococcal septicemia, not alone when the dose of infection approaches that used in the following experiments (0.08 cc. to 0.5 cc.), but also when it is 800 to 5,000 times smaller (0.0001 cc.).

Experiment 3. Combined Therapy.—(Table III.) Strain II 42[6]. Treatment: 0.45 cc. of a 2 per cent solution of ethylhydrocuprein in oil, per 20 gm. of mouse, and 0.2 cc. of antipneumococcus serum II.

TABLE III.

Mouse No.	Dose of culture.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.
	cc.							
1	0.08	Well	Well	Well	Well	Well	Well	Well.
2	"	"	"	"	"	"	"	"
3	"	"	"	"	"	"	"	"
4	"	"	"	"	"	"	"	"
5	"	"	"	"	"	"	"	"
6	"	"	"	"	"	"	"	"
7	"	"	"	"	"	"	"	"
8	"	"	"	"	"	"	"	"
9	"	"	X					
10	"	"	Well	"	■			
11	0.1	"	"	"	Well	"	"	"
12	"	"	"	"	"	"	"	"
13	"	"	"	"	"	"	"	"
14	"	"	"	"	"	"	"	"
15	0.2	"	"	"	"	"	"	"
16	"	"	"	"	"	"	"	"
17	"	"	"	"	"	"	"	■
18	"	"	"	■				

Experiment 3 a. Controls to Experiment 3.—Table IV shows (1) controls infected, and treated with 0.2 cc. of antipneumococcus serum alone; (2) controls of virulence.[1]

TABLE IV.

(1) Controls treated with 0.2 cc. of antipneumococcus serum alone.			(2) Controls of virulence.		
Mouse No.	Dose of culture.	Result.	Mouse No.	Dose of culture.	Result.
	cc.			*cc.*	
1	0.06	■ 24 hrs.	1	0.00001	■ 48 hrs.
2	"	■ 48 "	2	0.000001	■ " "
3	0.08	■ " "	3	"	■ " "
4	"	■ 24 "	4	"	■ " "
5	"	■ " "	5	"	■ " "
6	0.1	■ " "			

[1] For controls treated with ethylhydrocuprein see Experiment 2.

Comment on Experiments 3 and 3 a.—8 of 10 animals infected with 0.08 cc. of culture and treated with 0.45 cc. of the 2 per cent solution of the drug and 0.2 cc. of serum (each of which of itself is without effect with this dose of culture) survived. One animal died on the third day and pneumococci were not recovered from the heart's blood. The remaining animal died of pneumococcal septicemia on the fifth day (delayed death). All the mice infected with 0.1 cc. of culture and similarly treated survived; 50 per cent of the series infected with 0.2 cc. of culture and also similarly treated died of pneumococcal septicemia; the others survived. The control animals all died of pneumococcal septicemia within forty-eight hours.

Experiment 4. Combined Therapy.—(Table V.) Strain II 43[3]. Treatment: 0.45 cc. of a 2 per cent solution of ethylhydrocuprein in oil per 20 gm. of mouse, and 0.2 cc. of antipneumococcus serum II.

TABLE V.

Mouse No.	Dose of culture.	2d day.	3d day.	4th day.	5th day.	6th day.
	cc.					
1	0.3	Well	Well	■		
2	"	"	"	■		
3	"	"	"	■		
4	"	"	"	■		
5	"	"	"	■		
6	"	Sick	+ (eaten)			
7	0.5	Well	Well	Well	Well	Well.
8	"	"	"	"	"	"
9	"	"	+ (eaten)			
10	"	■				
11	0.7	Well	Well	"	"	"
12	"	"	■			
13	0.8	"	Well	Sick	■	
14	"	"	"	■		

Comment on Experiment 4.—In this experiment in which a larger dose of infection was given than in Experiment 3 the results did not come out uniformly or clear. The majority of the animals died, although in most cases death was delayed. We thought that a small dose of optochin given on the second day might serve to tide the animals over the third and fourth days and so help ultimate re-

covery; the result of this modification in dosage of the drug is shown in the next experiment. Virulence controls were done, as in Experiment 3 a, but are not shown in the table.

Experiment 5. Combined Therapy.—(Table VI.) Strain II 43[6]. Treatment: 0.45 cc. of 2 per cent ethylhydrocuprein in oil immediately after infection and 0.4 cc. after 24 hours' interval per 20 gm. of mouse; 0.2 cc. of antipneumococcus serum II at the time of infection.

TABLE VI.

Mouse No.	Dose of culture.	2d day.	3d day.	4th day.	5th day.	6th day.
	cc.					
1	0.3	Well	Well	Well	Well	Well.
2	"	"	"	"	"	"
3	"	"	"	"	"	"
4	"	"	"	"	"	"
5	"	"	"	"	"	"
6	"	"	"	"	■	
7	"	"	"	"	X	
8	"	"	"	■		
9	"	Sick	+ (eaten)			
10	0.5	Well	Well	Well	Well	"
11	"	"	"	"	"	"
12	"	"	"	"	■	
13	"	"	"	"	■	
14	"	"	"	■		
15	"	"	"	■		
16	"	"	Sick	■		
17	"	Sick	+ (eaten)			

Comment on Experiment 5.—50 per cent of the animals infected with 0.3 cc. of culture, and treated as stated, survived; of those that died, pneumococci were not recovered from the heart's blood of one; one was eaten by its fellows and could not, therefore, be autopsied; two gave positive blood cultures. In the case of infection with 0.5 cc. of culture, only two animals recovered; pneumococci were recovered from the heart's blood of all those that died except one, the body of which was so far eaten that it could not be bacteriologically examined. In this experiment the altered dosage of the drug ensured a better result than in the former case, but it is evident that we are at the limit of protective power for the conditions obtaining therein. Consequently we proceeded to study the effect of a single larger dose of drug combined with the serum therapy; namely, 0.5 cc. of the 2 per cent oily solution per 20 grams of mouse. Virulence controls were done, as in Experiment 3 a, but are not shown in the table.

Experiment 6. Combined Therapy.—(Table VII.) Strain II 44⁴. Treatment: 0.5 cc. of 2 per cent ethylhydrocuprein base in oil per 20 gm. of mouse, and 0.2 cc. of antipneumococcus serum II.

TABLE VII.

Mouse No.	Dose of culture.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.
	cc.							
1	0.2	Well	Well	Well	Well	Well	Well	Well.
2	"	"	"	"	"	"	"	"
3	"	"	"	"	"	"	"	"
4	"	"	"	"	"	"	"	"
5	"	"	"	"	"	"	"	"
6	"	"	"	"	"	"	"	"
7	"	"	"	"	+ (eaten)			
8	"	+ (eaten)						
9	0.3	Well	"	"	Well	"	"	"
10	"	"	"	"	"	"	"	"
11	"	"	"	"	"	"	"	"
12	"	"	"	"	"	"	"	"
13	"	"	"	"	"	"	"	"
14	"	"	"	"	"	"	"	"
15	"	"	"	+ (eaten)				
16	"	"	"	" "				
17	0.4	"	"	Well	"	"	"	"
18	"	"	"	"	"	"	"	"
19	"	"	"	"	"	"	"	"
20	"	"	"	"	"	"	"	"
21	"	"	"	"	"	"	"	"
22	"	"	"	"	"	"	"	"
23	"	"	"	"	"	"	+ (eaten)	
24	"	"	"	X				
25	"	■						

Two mice infected with 0.000001 cc. and one with 0.00001 cc. of Strain II 44⁴ (controls of virulence) died of pneumococcal septicemia in thirty-six hours. Thirteen controls infected with 0.08 cc. of culture and treated with 0.5 cc. of the ethylhydrocuprein solution alone immediately after the infection all died on the third day (forty-eight hours after infection) from pneumococcal septicemia.

Comment on Experiment 6.—In this case we increased the single dose of ethylhydrocuprein to 0.5 cc. of the 2 per cent solution of the base in oil per 20 grams of mouse. Six of eight animals infected with 0.2 cc. of culture, and six of eight animals infected with 0.3 cc. of culture, and treated as stated, survived. The four mice which died were eaten by their fellows and therefore could not be autopsied; in three of these cases death was delayed (fourth and fifth

days). Of the nine animals infected with 0.4 cc. of culture, six survived; one died on the second day with a sterile heart's blood (abundant inoculation) but pneumococci were recovered from the peritoneal cavity; one died on the fourth day and showed a sterile heart's blood on cultivation; and one was eaten by its fellows on the seventh day. The thirteen controls infected with 0.08 cc. of culture and treated with the dose of drug used in the actual experiment all died in forty-eight hours, pneumococci being present in the heart's blood of all. These controls are not shown in the protocol.

Experiment 7. Combined Therapy.—(Table VIII.) Strain II 45[8]. Treatment: 0.5 cc. of 2 per cent solution of ethylhydrocuprein base in oil per 20 gm. of mouse, and 0.2 cc. of antipneumococcus serum II.

TABLE VIII.

Mouse No.	Dose of culture.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.
	cc.							
1	0.4	Well	Well	Well	Well	Well	Well	Well.
2	"	"	"	"	"	"	"	"
3	"	"	"	"	"	"	"	"
4	"	"	"	"	"	"	"	"
5	0.5	"	"	"	"	"	"	"
6	"	"	"	"	"	"	"	"
7	"	"	"	"	"	"	"	"
8	"	"	"	"	"	"	"	"
9	"	"	"	"	"	"	"	"
10	"	Sick	"	"	"	"	"	"
11	"	"	"	"	"	"	"	"
12	"	"	"	■				
13	0.6	"	"	Well	"	"	"	"
14	"	"	"	"	"	"	"	"
15	"	"	"	"	"	"	"	"
16	"	Sick	Sick	Sick	■			
17	"	Well	■					
18	"	Sick	■					

Three virulence controls (0.000001 cc.) all died in forty-eight hours of pneumococcal septicemia.

Comment on Experiment 7.—All the animals infected with 0.4 cc. of culture and treated, and all but one infected with 0.5 cc. of culture and treated, survived; from the heart's blood of the one which died (fourth day) pneumococci were recovered. 50 per cent of those infected with 0.6 cc. of culture and treated survived; the

remainder died of pneumococcal septicemia (third tc fifth day). We have here, evidently, reached the limit of protection to be gotten by the simultaneous administration of antiserum and drug in the manner and quantities indicated above.

In addition to the experiment mentioned above, we have carried out a series of experiments in which the mice were treated with ethylhydrocuprein and a non-homologous serum. For example, we have used an antiserum to pneumococcus of Type I in the case of infection with from 0.0001 cc. to 0.1 cc. of a broth culture of a virulent pneumococcus of Type II (Strain II 34^{20}), the minimal lethal dose of which was 0.000001 cc.; and we have given the ethylhydrocuprein solution both as one dose (0.5 cc.) at the time of infection, and as three doses on the three first days of the experiment (0.25 cc., 0.25 cc., and 0.2 cc.), the administration of the first dose following the infection immediately; by thus combining the drug treatment with non-homologous serum therapy, we have seen no effect such as we have described above when the serum was homologous. Again, in the case of treatment of infection with a virulent strain of Group IV (Strain IV A 67.11^{3}), the minimal lethal dose of which was 0.000001 cc., we have had no such success on giving the drug and a potent antiserum to pneumococci of either Group I or Group II. The protocols of these experiments need not be exhibited.

DISCUSSION OF RESULTS.

The results of this study of the treatment of experimental pneumococcal infection in the mouse by simultaneous administration of ethylhydrocuprein and antipneumococcus serum are clear. The protocols show that 0.5 cc. of a 2 per cent oily solution of ethylhydrocuprein (optochin base), or 0.2 cc. of the type homologous antiserum, are each, by themselves, powerless to protect against 0.06 cc. of a highly virulent culture of a pneumococcus of Group II; but that if both these bodies, which are chemically far removed from each other, be exhibited simultaneously, protection is given to the animal against as large a dose of infection as 0.5 cc. of culture. The potency of the antiserum of Type II is such that 0.2 cc. confers a certain protection on mice against simultaneous infection with an amount of a virulent culture lying somewhere between 0.001 cc. to

0.01 cc.; with a higher dosage of culture this protection is quite uncertain, while it is altogether absent in the case of infection with 0.06 cc. of culture. The dose of ethylhydrocuprein generally used in the present study (0.5 cc. of the 2 per cent solution) is unable to protect against a dose of infection as small as 0.0001 cc. of a virulent culture. Hence, the exhibition of such a small dose of this drug as 0.5 cc. of the 2 per cent solution (0.01 of a gram) per 20 grams of mouse is capable of raising the threshold value of the serum more than fifty times; that is to say, mice are protected against 0.5 cc. of a virulent culture by the combined therapy, while 0.2 cc. of serum alone will not invariably protect against 0.01 cc. of culture, and the dose of ethylhydrocuprein used does not even protect against five thousand times less than 0.5 cc. of culture. Thus the protective value of simultaneous administration of the drug and serum, even though they be given by different routes, is many times greater than,

TABLE IX.

Combined Ethylhydrocuprein and Serum Therapy.

Type II Antiserum. Pneumococcus, Stock Strain of Group II.

Culture.	Ethylhydrocuprein 2 per cent solution.	Antiserum II.	Normal horse serum.	Survivals.	Deaths due to pneumococcal septicemia.	Deaths due to doubtful cause (no autopsy).
cc.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
0.001	—	0.2	—	100	0	0
0.01	—	"	—	66.6	33.3	0
0.02	—	"	—	40	60	0
0.04	—	"	—	60	40	0
0.06	—	"	—	0	100	0
0.08	—	"	—	0	100	0
0.08	0.45	—	—	0	100	0
"	"	—	0.2	0	100	0
"	0.5	—	—	0	100	0
"	"	—	0.2	0	100	0
0.0001	"	—	—	0	100	0
0.08	0.45	0.2	—	80	10	10
0.1	"	"	—	100	0	0
0.2	"	"	—	50	50	0
0.3	"	"	—	0	100	0
0.2	0.5	0.2	—	75	0	25
0.3	"	"	—	75	0	25
0.4	"	"	—	76.9	7.6	15.3
0.5	"	"	—	87.5	12.5	0
0.6	"	"	—	50	50	0

and out of all proportion to, the protective value of either alone. The preceding epitome of the results (Table IX) will make this clear.

It is important to note that, where a non-homologous antiserum is used in this combined method, *e. g.*, a Type I antiserum with a pneumococcus of Group II or a Type I or II antiserum with a pneumococcus of Group IV, no such effect as that just stated is seen. Indeed, no more effect is obtained than if normal, and not immune, serum be used. It seems that, if the dose of ethylhydrocuprein given in the first instance be below a certain quantity in relation to the amount of the infection, subsequent readministration of the drug on the next day is powerless to prevent a fatal result from pneumococcal septicemia.

CONCLUSIONS.

1. A single small dose of ethylhydrocuprein (optochin base), which by itself has practically no protective effect against experimental pneumococcal infection in mice, is capable of increasing the threshold value of the type homologous antipneumococcus serum at least fifty times.

2. This effect is proportionately many times greater than a simple summation of the protective effects of these two bodies.

3. No such effect is obtained when the antiserum used is one produced against a strain of pneumococcus from a group other than that to which the infecting pneumococcus belongs.

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THE MECHANISM OF ANAPHYLACTIC SHOCK.

Studies on Ferment Action. XXIII.

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Despite the intensive study which the phenomena of anaphylaxis have received, especially by immunologists, with considerable theorizing about the probability of protein splitting, and the source and the mechanism of production of the intoxicating agent, there have been published few experiments dealing with the actual ferment changes and the amounts of protein split products in the serum during shock.

Abderhalden and Pincussohn and their various coworkers (1) noted by means of the polariscope an increase in the power of the serum of sensitized animals to split the specific antigen. Pfeiffer and Mita (2) worked along similar lines. A summary of their work, together with detailed experiments carried out by means of the dialysis method, is found in a paper of Pfeiffer and Jarisch (3).

Auer and Van Slyke (4) have reported experiments in which they determined the amino-acid content of lungs of guinea pigs before and during shock. They found no noteworthy change.

In a recent paper Zunz and György (5) have confirmed the findings of Abderhalden and of Pfeiffer and Mita. Following the primary injection they found a gradual increase in a protease for beef protein, reaching a maximum in about 15 days; this ferment, according to their observations, disappears following shock, only to reappear on a further injection if made from 4 to 10 days after recovery from shock. They definitely determined an increase in amino-acids during acute shock in dogs, this being quite contrary to the supposition of de Waele (6).

In previous papers we have shown that the homologous serum may be toxic for animals under conditions which remove the antiferment and permit serum protease to act (7); that anaphylatoxin formation depends upon the adsorption of the serum antiferment (8); while an increase in antiferment tends to diminish the susceptibility to shock (9). During trypsin shock, which closely resembles anaphylactic shock, we have noted a marked increase in the serum protease and lipase (10).

The present experiments deal with the serum ferments and the protein split products during anaphylactic shock in dogs. The changes that occur are striking and have not, so far as we are aware, been recorded by other workers. We believe that the facts developed confirm the idea of a protein cleavage, in contradistinction to the supposition of a purely physical change, as advanced, for instance, by von Behring (11) and previous workers. But this cleavage concerns the proteins of the animal itself, rather than the antigen.

While these observations have been made on the serum, the deduction that the primary site of the reaction is to be found in the serum is not therefore warranted, for the serum changes may simply express the end results of cellular changes.

Technique.

Dogs have been used exclusively in our experiments, not only because of the larger amounts of serum which are available and which are needed in work of this kind, but because the greater susceptibility of the bronchial musculature of the guinea pig is lacking to a large degree, so that death from asphyxia does not complicate the picture before serum changes become established. The dog, too, probably more closely parallels the human condition. The methods used to determine the ferments and the split products have been fully described in a previous paper (12). The proteoses present in the serum have been determined as follows: 5 cc. of serum are diluted with about 10 cc. of normal saline and 2 cc. of a mixture of 10 per cent acetic acid and 20 per cent salt solution are added. The serum is then boiled for 10 minutes, filtered through hard paper, and the filtrate saturated while boiling with sodium sulphate. The precipitate is collected and after washing with water saturated with sodium sulphate it is dissolved from the filter paper by means of a small amount of water made slightly alkaline with sodium carbonate. The dissolved proteoses are collected directly in the large Jena glass tubes used for oxidizing, and nitrogen determinations are made. The results charted therefore represent the amount of nitrogen present as proteoses in 5 cc. of serum. The

majority of the determinations made to determine the amount of non-coagulable nitrogen in the serum, together with the amount of protease activity, have been made in duplicate.

Purified horse serum albumin has been used as an antigen. This preparation, containing antiferment, shows considerable resistance to tryptic digestion. Injections have been intravenous; blood samples have been obtained from ear veins; during shock recourse to a suction pump has been found necessary to obtain blood in sufficient quantity.

The Effect of Primary Injection and Sensitization.

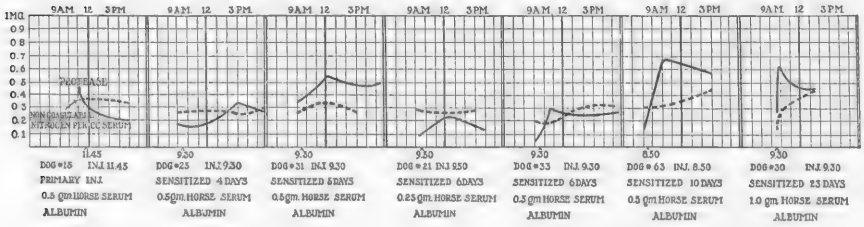
The first experiments illustrated the changes that occur in the serum following the first or sensitizing dose.

Dog 25.—Weight 8 kilos. Sensitized Feb. 27, 1915, and Mar. 2, 1915, with subcutaneous injections of horse serum albumin. On Mar. 3, 1915, at 9.30 a. m. 0.5 gm. of horse serum albumin was injected intravenously. There resulted an immediate rise in temperature to 106° F. at 11.10 a. m., together with an immediate leucopenia. The antiferment index was practically unaltered, as was also the amount of non-coagulable nitrogen in the serum. The serum protease showed no change until the afternoon, when a moderate increase was noted. The serum lipase increased during the same time. Blood drawn the following morning showed a slight increase in antiferment, a decrease in protease and lipase, and a slight increase in non-coagulable nitrogen.

Dog 21.—Weight 4 kilos. Sensitized in the same manner as Dog 25. 0.5 gm. horse serum albumin injected at 9.50 a. m., Mar. 5, 1915. The results are similar to those of the first animal, except that the rise in protease was noted a little sooner than in the previous dog. The non-coagulable nitrogen showed a considerable increase after 24 hours.

Inasmuch as the interest in anaphylaxis centers chiefly about the protease and the amount of split products, we have collected the results of a series of experiments dealing with the injections of the antigen at various intervals after sensitization in Text-fig. 1. From this it will be noted that there is a progressive decrease in the time interval of the mobilization of the protease in the serum. Thus in Dog 15, a single injection in a non-sensitized dog has resulted in a decrease in protease, and practically no change in the non-coagulable nitrogen. The dogs which received injections of the antigen from four to ten days after sensitization (Nos. 25, 31, 21, 33, and 63) showed an increase in protease at an interval after the in-

jection which becomes progressively less, while a rise in the non-coagulable nitrogen in the serum becomes evident in those animals which are sensitized longest. When the animal finally becomes fully sensitized the ferment mobilization is immediate, as is also the increase in non-coagulable nitrogen.



TEXT-FIG. 1. Mobilization of serum protease during the course of sensitization.

It should be emphasized that the ferment which we demonstrate is not specific, for when the serum is emulsified with chloroform and incubated, the ferment digests the serum proteins. The only evidence of specificity lies in the fact that the ferment is mobilized by the injection of the specific antigen.

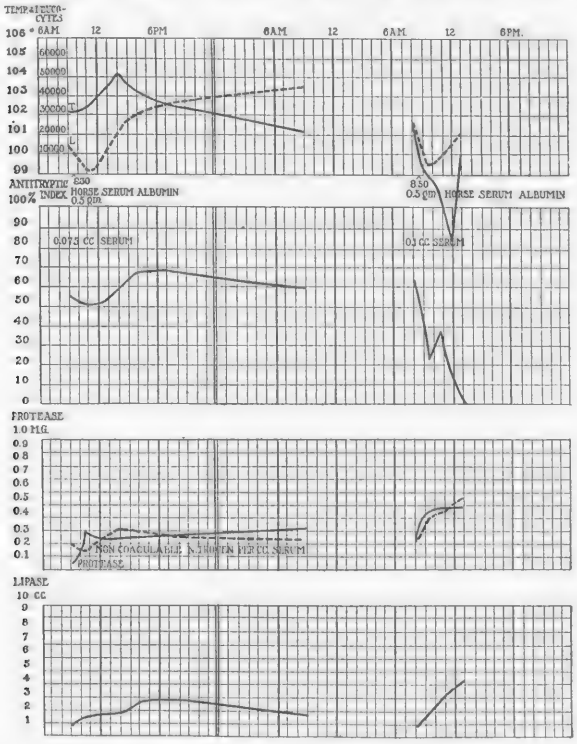
The Effect of Acute Anaphylactic Shock.

The following experiments serve to illustrate the effect of the reinjection of the antigen after complete sensitization.

Dog 15.—Weight 4.5 kilos. Sensitized Feb. 9, 1915, with 1.0 gm. of horse serum albumin intravenously. The primary injection resulted in the usual rise in temperature and fall in leucocytes. The antiferment, which had a relatively high titer, fell slightly, then made a gradual recovery. The non-coagulable nitrogen showed practically no change, while there was a decided fall in the protease content. The lipase showed a gradual increase from 1.5 to 5 cc. N/100 sodium hydrate after 24 hours. When we now contrast this picture with that following the injection after sensitization, there has been noted a much greater fall in the antiferment titer, an immediate rise in non-coagulable nitrogen (from 0.27 to 0.45 mg.), together with a large increase in serum protease (from 0.47 to 0.87 mg.). The lipase increased from 0.8 to 6.8 cc. The animal died 4 hours after the injection.

Dog 33.—Weight 9 kilos. Sensitized, beginning Mar. 8, 1915, with three subcutaneous injections of horse serum albumin. The effect of the primary in-

travenous injection before complete sensitization is shown in Text-fig. 2. The injection (0.5 gm. of horse serum albumin) was made at 9 a. m., Mar. 15, 1915 (7 days after the first sensitizing dose). As a result there was noted a distinct rise in the protease, a slight increase in the lipase in the afternoon; a slight fall,



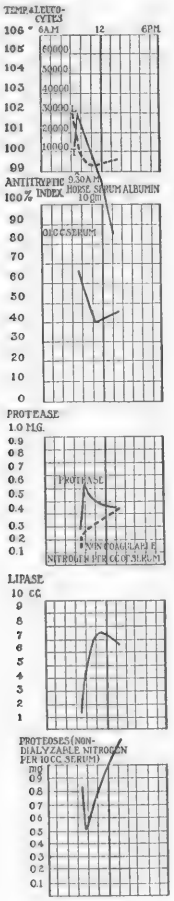
TEXT-FIG. 2. TEXT-FIG. 2 a.

TEXT-FIGS. 2 AND 2 a. Serum changes following antigen injection before and after complete sensitization.

followed by a rise in the antiferment titer; the non-coagulable nitrogen first showed a slight fall followed by a rise in the afternoon. The intoxicating injection was made Mar. 29, 1915, at 8.50 a. m. (0.5 gm.) (Text-Fig. 2 a). As a result there was a typical fall in the antiferment titer, and an immediate increase in the protease, lipase, and non-coagulable nitrogen. The animal died at 1.30 p. m. Of the total amount of the non-coagulable nitrogen in the blood serum at this time 33 per cent represented nitrogen present in the form of proteoses. It is possible that some of these proteoses may be toxic.

Dog 30.—Weight 7.7 kilos. Sensitized Mar. 7, 1915. Reinjected at 9.30 a. m.,

Mar. 30, 1915, with 1.0 gm. horse serum albumin. Died at 1.30 p. m. (Text-fig. 3). In this experiment a larger amount of antigen was injected for the intoxicating dose than in the two previous animals. The ferment picture is typical



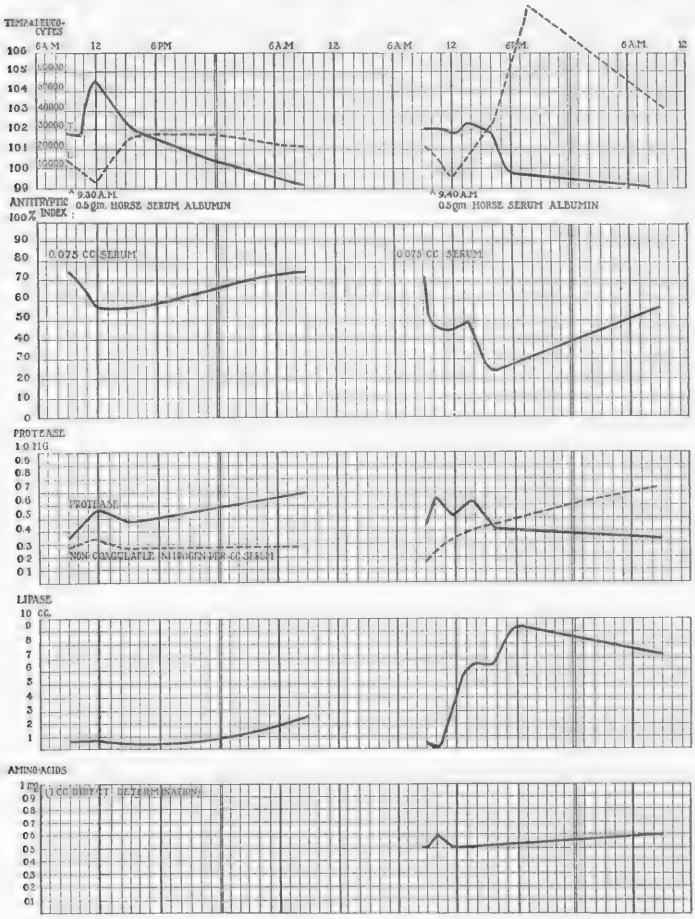
TEXT-FIG. 3. Serum changes during acute anaphylactic shock.

and similar to the other experiments. In this animal the amount of proteoses present in the serum was determined by dialyzing the filtrate from 10 cc. of serum after the coagulable nitrogen had been removed by acid and heat. Nitrogen determinations were then made on the undialyzable portion. This represents

the higher split products (proteoses). As will be observed from Text-fig. 3, there resulted an almost immediate drop in the amount present in the serum with an increase following later.

Protracted Shock.

The following experiments illustrate the conditions when the animal does not die within a few hours, but lives over a period of about twenty-four hours following the second injection.



TEXT-FIG. 4. TEXT-FIG. 4 a.

TEXT-FIGS. 4 AND 4 a. Serum changes during protracted anaphylactic shock.

Dog 31.—Weight 11 kilos. Sensitized Mar. 7, 1915. First intravenous injection at 9.30 a. m., Mar. 12, 1915 (Text-fig. 4). On Mar. 30, 1915, at 9.40 the second intravenous injection was made. The dog showed the usual malaise and prostration, but apparently recovered somewhat in the afternoon. The animal died at 8.30 the following morning. As will be observed from Text-fig. 4 a there was noted a marked leucocytosis (97,000) in the evening. The antiferment showed the usual fall with a recovery the next morning. The non-coagulable nitrogen increased from 0.17 mg. per cc. to 0.75 mg. the following morning. The protease showed an immediate increase with some fluctuations following, and a gradual decline. The lipase increased to a marked extent. The amino-acids (Van Slyke method) increased immediately after the injection, then fell to the original figure, and increased the following morning. Whatever may have been the factor which delayed exitus in this dog, it seems to have had some influence on the ferments and the antiferments, and was manifested possibly first in the fall in protease at noon. It will be observed that all the curves in the experiment are broken and in so far differ from the normal course of events during shock.

The Effect of Reinjection During the Refractory Stage.

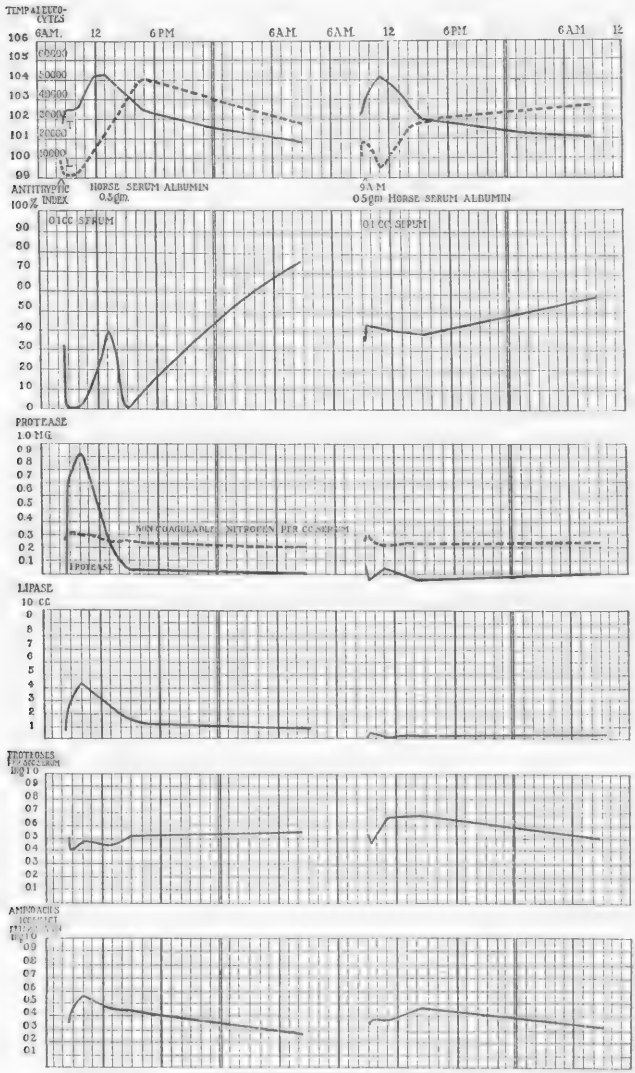
In the following experiment the effect of a single shock in the sensitized animal, followed by a second injection after two days, has been studied.

Dog 65.—Weight 6.2 kilos. Sensitized by an intravenous injection, Apr. 2, 1915. On Apr. 20, 1915, at 8.37 a. m., 0.5 gm. of horse serum albumin was injected, which dose was sufficient to cause a profound intoxication, but not death. The animal was bled at 8.50 and 10.00 a. m., 1, and 3 p. m., and the following morning. The resulting serum changes are quite typical (Text-fig. 5). The antiferment showed the usual immediate drop, with a following recovery and second drop. The non-coagulable nitrogen showed only a slight increase and remained low, thus differing from the other animals so far studied. The rise in protease was instantaneous and very marked, fell rather rapidly, and then remained low. The lipase showed the usual rise, but not to such a marked extent. The serum proteoses showed the immediate diminution which is characteristic, while the amino-acids increased quite markedly.

On reinjection two days later (9 a. m., Apr. 22, 1915) it will be noted (Text-fig. 5 a that the change in antiferment was absent. The protease showed some decrease and remained low, as did the lipase. The proteoses showed a slight decrease after 15 minutes with a distinct increase later. The amino-acid increased gradually and returned to normal the following morning.

Shock in the Pregnant Animal.

During pregnancy animals are known to show a relative immunity to anaphylactic shock; the mechanism of the resistance is not understood. It is possible that the increase in antiferment which usually accompanies pregnancy might in a measure account for this



TEXT-FIG. 5. TEXT-FIG. 5 a.

TEXT-FIGS. 5 AND 5 a. Serum changes during acute anaphylactic shock and following antigen injection during the refractory period.

resistance, since a certain amount of resistance can be imparted to an animal by increasing its antiferment, as we have shown previously (9).

The following experiment was made on a pregnant animal.

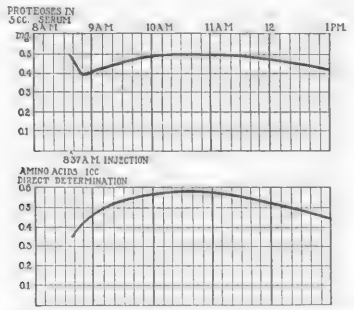
Dog 56.—Weight 6 kilos. Sensitized by an intravenous injection of horse serum albumin Apr. 2, 1915. A second injection, made three days later, resulted in no marked alterations with the exception of a rather sharp drop in the afternoon in the amount of proteoses contained in the serum. The intoxicating dose was made at 8.30 a. m., Apr. 22, blood being collected at 8.45 and 9.30 a. m., 12, and 3 p. m. The animal was somewhat nauseated and vomited several times, but remained in fair condition until the afternoon; it died suddenly at 4.30 p. m. In the first place it was observed that the antiferment instead of being high was quite low (0.15 cc. of serum inhibiting only about 10 per cent of the digestion). The protease, however, rose sharply, as in the other experiments, although there was no increase in non-coagulable nitrogen until towards noon. The lipase curve was very irregular, but continued to rise. The proteoses were practically unaltered for 15 minutes, later increased, decreased toward noon, and increased markedly toward the terminal stage. The amino-acids at first declined progressively, but increased during the afternoon.

The Relation of Serum Proteoses and Amino-Acids to Shock.

When the antigen is injected into the circulation of the sensitized animal proteolysis takes place. This fact has been definitely established through the work of Zunz and György in demonstrating an increase in amino-acids by the Van Slyke method during shock. The various experiments which we have detailed have also illustrated this phase, inasmuch as an increase in the non-coagulable nitrogen is an almost invariable accompaniment of the acute shock. The question arises whether Zunz and György and the other workers who have advanced the idea of specific protease action are justified in assuming that the increase in amino-acids proves that the specific antigen has been split.

Before the antigen reaches the amino-acid stage it must pass through the various cleavage phases,—proteoses, peptones, etc.,—and we should expect that there would be a preceding or concomitant increase in these split products in the serum. The contrary is true. This is illustrated in Text-fig. 6, in which the amino-acid and protease curves are illustrated immediately after the injection of the antigen at 8.37 a. m. (Dog 65). The immediate rise in

amino nitrogen is apparent, but is accompanied by an actual decrease of the amount of proteoses in the serum. We have repeatedly observed this relation and have never noted an increase in proteoses during the first fifteen minutes following the antigen injection,



TEXT-FIG. 6. Relation of serum proteoses and amino nitrogen during acute anaphylactic shock.

when the anaphylactic symptoms are, of course, already well advanced. The decrease of the proteoses at the instant of the shock, together with an increase in amino-acids, seems to warrant the assumption that the primary cleavage that occurs has its substrate in the proteoses already present in the serum.

Non-Specificity of Serum Protease.

In the preceding paragraph attention has been called to the fact that when the increase in amino-acids occurs immediately after the injection of the antigen into the sensitized animal, there is a simultaneous decrease in the higher split products of the serum, a condition which is contrary to the view that it is the injected antigen that is being split. For if that were true we should expect an increase in the higher split products, produced during the process of cleavage of the antigen to the amino-acid stage. The idea that it is the antigen that is split has seemed in accord with the data at hand up to the present time. Thus Abderhalden and Pincussohn and Pfeiffer and Mita report positive results when the serum of sensitized animals is permitted to act upon the specific antigen *in vitro*.

And the experiments of Zunz and György confirm these findings by means of the increase in amino-acids demonstrable with the Van Slyke apparatus *in vivo* and when the specific serum is permitted to "digest" the antigen *in vitro*. So, too, they also report that immediately following shock this property disappears, affording an attractive explanation for the state of anti-anaphylaxis. This entire hypothesis concerning the mechanism of anaphylactic shock is based, therefore, upon the specificity of the serum proteases. In a previous paper we have shown that there is no element of specificity in so far as the proteases are concerned in the digestion occurring in the Abderhalden reaction (13). The digest is obtained from the serum proteins and is brought about by changes in the colloidal state of dispersion of the serum induced by the antigen introduced as a substrate, resulting in what may be termed local areas of antiferment deficiency, due either to an actual adsorption of antiferment by a formed substance, such as a precipitate, or to a change in the degree of dispersion of the unsaturated lipoids upon which the antiferment property depends, as we have previously demonstrated (14). If a protease is present in such sera a positive reaction can occur; if not, the digestion does not take place.

Since it is well known from various immunological experiments that minimal amounts of the antigen can call forth a specific reaction, as, for instance, in the precipitin test or in complement deviation, we can, by means of dilution of the specific substrate, show that the amount of digestion brought about by the addition of such a specific substrate may be greater by many times than the total amount of nitrogen introduced, thus positively proving that the digestive products are not derived from the specific substrate and can only be formed from the serum itself. Such an experiment is shown as follows: Fresh serum is used from a fully sensitized dog. A 1 per cent horse serum albumin solution, to which the dog has been sensitized, is used as a substrate and varying dilutions to 0.001 per cent are made. If we now add a constant amount of the ferment (the specific serum) to decreasing dilutions of the substrate, we should expect that with minimal dilutions there would not be enough substrate present for the ferment to act upon, and as a result

the amount of digestion products should become smaller. In the following experiment the serum was allowed to "digest" the horse serum albumin solution for twenty hours under toluol (Table I).

TABLE I.

Tube No.	Serum 56/5.	Horse serum albumin solution.		Nitrogen available as substrate.	Amino-acids determined in 1 cc. of mixture (direct).	Increase in amino-acids.
	cc.	*cc.*	*per cent*	*mg.*	*mg.*	*mg.*
1	1	1	1	0.74	0.279	0.034
2	1 (inactivated)	1	1	0.74	0.245	
3	1	1	0.1	0.074	0.245	0.055
4	1 "	1	0.1	0.074	0.190	
5	1	1	0.01	0.0074	0.256	0.078
6	1 "	1	0.01	0.0074	0.178	
7	1	1	0.001	0.00074	0.268	0.078
8	1 "	1	0.001	0.00074	0.190	

As will be observed, with an available total nitrogen from the specific substrate of about 0.00074 mg. (Tubes 7 and 8) we obtain a digest of 0.078 mg. of amino-acids per cc. of the mixture. This experiment, which we have repeatedly made, affords absolute proof that the theory of specific protease action is without warrant. The various methods of demonstrating specific protease action all have a common error in that the digestion of the serum proteins and of the higher split products present in the serum (proteoses) by the non-specific serum proteases is ignored. The serum protease should be regarded as a simple ferment, polyvalent in character, and in so far resembling the ordinary tryptic ferment. This is precisely the view that Fermi (15), working with a variety of methods, has reached. Serum ereptase should, however, be strictly distinguished from the protease.

The Serum Esterase.

An invariable accompaniment of the shock has been a marked mobilization of the serum esterase. While the first injection of horse serum albumin may cause some increase in the ferment, it never causes so sudden nor so extensive a rise in the esterase curve. This increase is not specific for anaphylactic shock, but occurs following trypsin and peptone shock, as well as following intoxication by bacterial proteins of varied derivation. This increase prob-

ably represents a mobilization from tissue cells in general rather than the secretion of any one organ.

DISCUSSION.

In a previous paper (8) we stated:

"The arguments made against the protein-intoxication conception, such as the time element and the minute amount of substance necessary, are not convincing, for we know that ferment action may be very rapid; and as far as the quantity of substrate is concerned the argument fails if we place the matrix of the poison in the serum proteins themselves. There is some reason to believe, however, that if ferment action is the basis of anaphylactic shock, these ferments may have a much wider range of action than merely on the introduced protein."

The facts brought out during the course of this work, have, we believe, established this view as essentially correct. In brief, these results can be stated as follows: The injection of the antigen in the non-sensitized animal is practically without influence on the serum ferments or the split products present in the serum.

During the period following the sensitizing injection and preceding the development of complete sensitization the organism responds to the injected antigen by a progressively increasing rapidity of mobilization of ferments, the amount of protease mobilized becoming greater as the maximum of sensitization is reached. This protease is not specific.[1]

The acute shock is accompanied by an immediate and marked increase in serum protease; by a fall in antiferment; by a rise in the amino-acid content and non-coagulable nitrogen of the serum, together with a primary decrease in the serum proteoses. The esterase rise is constant and progressive. When death occurs after a few hours the serum may contain a large amount of proteoses (33 per cent of the total non-coagulable nitrogen).

Before discussing further the mechanism of the various phenomena we must emphasize the fact that whatever change takes place during the course of the reaction is not due to specific pro-

[1] In a paper which has appeared after the completion of our work Pfeiffer (Pfeiffer, H., *Ztschr. f. Immunitätsforsch., Orig.*, 1915, xxiii, 515) has demonstrated an increase in the peptolytic power of the serum during anaphylactic shock. Because of the extent of the paper it cannot be adequately reviewed at this time.

teases (*Abwehrfermente*). The antigen which we used (purified horse serum albumin) is exceedingly resistant to the ordinary tryptic ferment, a ferment which is not only polyvalent but also very active; and it seems unreasonable that this same protein should be readily attacked by a specific protease when we know that the antiferment, to which the horse serum albumin owes its resistance, inhibits not only the tryptic ferment but also the so called specific ferment. The actual digestion is due to an entirely different mechanism,—to a non-specific ferment acting upon serum proteins. This digestion is brought about through colloidal changes which take place when the antigen is brought into contact with the serum. Either a precipitate is formed which may act as an adsorbing substance for the antiferment, or else the dispersion of the antiferment itself is also altered, so that local areas of antiferment deficiency are formed with a resulting immediate cleavage either from the serum proteins (especially the globulins) or from the proteoses already present in the serum. Bearing these conditions in mind, the mechanism of the production of the toxic substances which produce the acute shock becomes relatively simple, but involves not only the serum, but the cellular elements of the organism as well, and the latter probably primarily.

The mobilization of the protease must be a cellular phenomenon and during the course of sensitization this response is increased not only in intensity, but also in rapidity. This need not result in an intoxication, for the isolated cell, as the various investigators who have studied cellular phenomena in anaphylaxis have shown (Dale, Schultz, Weil, etc.), may be capable of repeated response to specific stimuli; so we must concede that the antigen is not in itself toxic for the cell either before or after sensitization. Associated, however, with the cellular response we find serum changes that do result in an intoxication. There is an immediate fall in the antiferment titer. This may in part be due to a saturation of the antiferment by the mobilized ferment. It is more probably due to a colloidal change with a resulting lessening of the dispersion of the unsaturated lipoids upon which the antiferment property depends. This lessening of the antiferment titer will, of course, facilitate

proteolysis. Furthermore, definite changes in the antibodies of the serum (especially the precipitins) occur at the time of shock. Whether or not an actual change to the final picture which is demonstrable *in vitro* with the precipitin reaction occurs is immaterial. The precipitin content which before the shock is present in the serum disappears instantly when shock is induced (Joachimoglu (16)). There is evident at least a colloidal rearrangement which must result in local areas of antiferment adsorption or diminution in which proteolysis can take place.

This proteolysis is made evident by the immediate increase in amino-acids. The only question relates to the substrate from which the amino-acids are derived. Contained in the serum of normal guinea pigs and dogs one finds a relatively large amount of higher split products (5 cc. of serum containing from 0.3 to 0.5 mg. of nitrogen in this form). Immediately after shock these split products are diminished in amount, but later increase progressively until death takes place. It is quite evident that if we were dealing with a shock primarily due to protein split products derived from the introduced antigen we should expect to find an immediate increase in the higher split products derived from that antigen.

Briefly, then, the mechanism of acute intoxication may be stated as follows: An immediate mobilization of a non-specific protease in large amounts; the formation of a state of antiferment deficiency through colloidal changes; a simultaneous cleavage of serum proteins (proteoses) through the peptone stage to amino-acids; an intoxication by these peptones with a resulting cellular injury, made evident by an increase in serum lipase, the fall in temperature, and other manifestations of acute shock. The elements of specificity lie in the mobilization of the non-specific cellular ferment and the colloidal changes in the serum, not in the production of specific ferments.

In a later paper we shall discuss more fully the mechanism of anti-anaphylaxis and other manifestations of protein intoxication.

CONCLUSIONS.

1. The serum ferments are practically unaltered by a primary injection of foreign protein.

2. During the course of sensitization the injection of the antigen is followed by the mobilization of a non-specific protease which increases in rapidity and intensity as the maximum period of sensitization is reached.

3. Acute shock is accompanied by:

(a) The instantaneous mobilization of a large amount of non-specific protease; (b) a decrease in antiferment; (c) an increase in non-coagulable nitrogen of the serum; (d) an increase in amino-acids; (e) a primary decrease in serum proteoses.

4. Later there is a progressive increase in the non-coagulable nitrogen, in proteoses, and in serum lipase.

5. The acute intoxication is brought about by the cleavage of serum proteins (and proteoses) through the peptone stage by a non-specific protease.

6. The specific elements lie in the rapid mobilization of this ferment and the colloidal serum changes which bring about the change in antiferment titer.

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A CONTRIBUTION TO THE BIOLOGY OF PERIPHERAL NERVES IN TRANSPLANTATION.[1]

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PLATES 44 TO 46.

(Received for publication, May 11, 1915.)

The large amount of experimental and clinical work in transplantation that has been done during the last few years has elucidated many essential points, and the transplantation of bone, skin, connective tissue, blood vessels, and glandular organs has assumed a practical value. This is not the case with peripheral nerves, possibly because their transplantation in human beings has been mainly heteroplastic.

The processes leading to the degeneration of a divided peripheral nerve are well known, and the work of Nageotte has recently added minute and important histological details to our knowledge of the cells of Schwann and the part played by them in the degeneration and regeneration of a peripheral nerve. I shall not comment upon attempts at transplantation of nerves in human beings. Of the experimental work in this field I shall mention only the results of those investigators who have made histological examinations of the transplanted pieces, especially for the Wallerian degeneration, and who have attempted, in the case of a negative result, to determine the variations in these processes; that is, in the Wallerian degeneration, produced by transplantation.

Huber[2] has examined histologically mainly heteroplastic transplanted segments of nerves, and concluded from his experiments that the degenerative process, which occurred in these pieces was very much like the true Wallerian degeneration, the various stages of which, however, succeeded each other more rapidly in the graft than in the peripheral part of a divided nerve.

[1] Aided by a grant from The Rockefeller Institute for Medical Research.

[2] Huber, G. C., *Jour. Morphol.*, 1895, xi, 629.

Ballance and Stewart[3] in their experiments on transplantation are reserved in their conclusions, expressing themselves as follows: The degeneration appears in the graft exactly as in the peripheral part of a divided nerve. The graft itself is a piece of dead tissue and is gradually absorbed and replaced like a clot by living tissue. The regeneration occurs later, but is not a result of the work of the cells of the graft itself. This conclusion seems to me to express two conflicting views, of which one, if true, necessarily excludes the other. If the degeneration of the graft appears exactly as in the peripheral segment of a divided nerve, then the segment is not dead. For the peripheral segment of a divided nerve is not dead. The axis cylinder dies and degenerates, but the cells of Schwann, on the other hand, after the division give evidence of their life by their proliferation.

Neither Ballance and Stewart nor Huber discriminated between the auto-, homo-, and heteroplastic transplantation. This was first done by Merzbacher.[4] In 1905 Merzbacher observed that the graft in auto- and homoplastic transplantations only degenerated in a typical way, whereas in heteroplastic transplantations there occurred in the fibers various regressive processes that resulted in the necrosis of the piece. In auto- and homotransplantations the grafts survive and therefore are capable of a true Wallerian degeneration, which can take place only in nerves in a condition of survival. In heterotransplantations the grafts die and cannot degenerate, but become necrotic; in these cases there is no, or only an insignificant formation of myelin ovoids. The conclusions of Merzbacher were confirmed by the experiments of Segale.[5]

Verga[6] in 1910 performed a series of homo- and heterotransplantations, bridging the central and peripheral part of a divided nerve by the graft. Verga found that the segments healed and always degenerated, and that from an anatomical standpoint there was no difference between the homo- and the heteroplastic graft.[7]

In 1911 Maccabruni[8] in Golgi's laboratory made a number of homo- and heteroplastic transplantations. His results are in accord with those of Huber and Verga, recording a typical Wallerian degeneration in homoplastic as well as in heteroplastic grafts. The center of both kinds of graft he found necrotic, possibly due to the lacking supply of nourishment. Thin grafts degenerated completely without necrosis. From the 8th to the 14th day he found proliferation of the cells inside the nerve fibers by means of karyokinetic division. The process has a slower course than in a degenerating nerve. Regarding the origin of the cellular elements inside the fibers, Maccabruni expresses some reservation. His preparations do not show whether these cells represent the syncytium of Schwann, or whether they are connective tissue cells.

[3] Ballance, C. A., and Stewart, P., *Rev. neurol.*, 1902, x, 860.

[4] Merzbacher, *Neurol. Centralbl.*, 1905, xxiv, 150.

[5] Segale, L., cited by Maccabruni, F., *Folia neuro-biol.*, 1911, v, 598.

[6] Verga, *Jahresber. f. Chir.*, 1910, xvi, 481.

[7] This publication is not available. In the summary of the article in the *Jahresber. f. Chir.* the cells of Schwann are not mentioned.

[8] Maccabruni, *loc. cit.*

Besides the work just mentioned, experimental transplantation of nerves has been performed by Gluck,[9] Kilvington,[10] and Duroux.[11] The results of these investigators are encouraging as far as the function is concerned. Neither Kilvington nor Duroux, however, made histological examinations of the transplanted pieces, and the interpretation and the conclusions drawn from his material by Gluck concerning the processes of regeneration are not convincing. The lack of proof, in the work of Gluck, that the graft is different from dead material has caused Kölliker to remark that the nerve bridge must be supposed to prevent the regeneration of the peripheral part instead of facilitating it, and Kölliker advocates the bridging of the defect by strands of catgut or tubes as superior to the application of a graft of nervous tissue.

In the problem of transplantation of nerves the question of the fate and survival and multiplication of the cells of Schwann is of importance. The solution of this point, which is the only reliable sign of the survival of the transplanted piece, gives the key to the problem and will influence the procedure of surgeons in cases of nerve defects. If the grafts die and become necrotic they are no more suitable for bridges than strands of catgut. If it is true, on the other hand, that the grafts do survive, the statement of Kölliker lacks support, and in bridging nerve defects grafts of peripheral nerves must be preferred to dead material.

I have experimented on the sciatic nerve of rabbits, from which pieces 2 to 3 cm. long are taken out and then either reimplanted into the same animal, united to the cut ends of the nerve by means of a single silk suture (autoplastic), or implanted into the sciatic nerve of another rabbit (homoplastic), or into guinea pigs (heteroplastic). I have made three series of experiments. In each series I have operated on several animals and the transplanted pieces were removed for histological examination at different intervals (4, 8, 12, 16, or 20 days) after the transplantation.

Then the pieces were treated in the way indicated by Nageotte, which has given excellent results in his study of the Wallerian degeneration. The grafts were hardened in Dominici's solution, next they were dissociated by means of needles as far as possible, and stained by hematoxylin before passing through alcohol and mounted in cedar oil. From some cases sections were prepared and treated according to the method of Marchi.

[9] Gluck, *Jahresber. f. Chir.*, 1895, i, 282.
[10] Kilvington, B., *Brit. Med. Jour.*, 1908, i, 1414.
[11] Duroux, E., *Lyon Chir.*, 1912, viii, 562.

I wish to emphasize the necessity of making dissociation preparations, if one wishes to make indisputable observations on the cells of Schwann. In such preparations only we are sure that a certain cell belongs to a certain fiber, and in this case only can we count the accurate number of cells in each individual fiber. I shall give a summary of my results from each of the three series, beginning with the autoplastic transplantations.

Autoplastic Transplantations.

In this series the examination of the graft four and six days after transplantation (Fig. 4) reveals a process which is not very different from the ordinary Wallerian degeneration (Figs. 1, 2, and 3). The nuclei of the cells of Schwann have fallen in towards the center of the fibers between two myelin ovoids. The nuclei are richly provided with chromatin and are embedded in protoplasm. In a few fibers from the sixth day two or three nuclei are observed close to each other, indicating that multiplication of these cells has already begun. Between and in the myelin ovoids there are immigrated mononuclear cells of a type quite different from the cells of Schwann. The nuclei of these cells are smaller and richer in chromatin than the nuclei of the cells of Schwann. They present phagocytic properties filling their cell bodies with fragments of myelin, and Nageotte, who found them in the Wallerian degeneration, called them "*corps granuleux.*" Probably they are lymphocytes.

The only difference between the autoplastic graft from the fourth and sixth days and the peripheral part of a divided nerve is that in the latter the formation of myelin ovoids is more advanced than in the graft. In the examination of the grafts in later stages, we find this feature again and again. After eight days we find a degenerative process resembling a somewhat delayed Wallerian degeneration. A graft from the eighth day is in as degenerative a stage as a peripheral nerve from the fifth to sixth days. But in some fibers we observe no degeneration at all; there is no formation of myelin ovoids and they look perfectly normal. These fibers belong to the central parts of the graft, and judging from the appearance

of Marchi preparations, these fibers later become necrotic. This is true about the central fibers of homoplastic grafts as well as autoplastic. On the twelfth day we find as pronounced a Wallerian degeneration as on the eighth or ninth day. A large number of myelin ovoids has been formed, and there are many "*corps granuleux*" and numerous nuclei of the cells of Schwann.

After the sixteenth and twentieth days (Figs. 5 and 6) the nuclei of the cells of Schwann are arranged in long rows inside the sheaths of Schwann with continuous protoplasmic bridges about and between them; the only difference from the Wallerian degeneration of the same stage is that in the latter the absorption of myelin fragments is a good deal more advanced than in the graft.

Homoplastic Transplantations.

I shall next describe the results of my homoplastic transplantations.

In almost every respect the preparations from the fourth and fifth days (Figs. 7 and 8) resemble the picture of a nerve on the fourth and fifth days of Wallerian degeneration. The nuclei of the cells of Schwann have fallen in towards the center of the fibers, and are richly provided with chromatin. A formation of myelin ovoids has started and only a few immigrated cells are seen. In one of the preparations from the fourth day we find a cell of particular interest. In one of the fibers (Fig. 8) we observe three oval nuclei of Schwann close to each other, and also a large darkly stained cell including a nucleus in mitotic division. This cell gives proof that the cells of Schwann multiply in a homoplastic transplanted graft. The increased number and the long rows of nuclei of Schwann in individual fibers already give strong evidence for the probability of such a conclusion, but the mitotic figure presents a picture from a stage of the process itself. We do not hesitate in the identification of this cell. The well outlined protoplasmic body of the cell, which is never observed in the immigrated cells during their mitotic division, determines that the cell is really a cell of Schwann.

I have never observed in the grafts in autoplastic transplantation such a mitotic division of the cells of Schwann. But mitotic divi-

sions are not easily found and are not frequently seen in the cells of Schwann during their proliferation in the ordinary Wallerian degeneration. Accordingly there is no reason to doubt that mitotic divisions may also be observed in autoplastic transplantations, since we know that grafts in such conditions are best fit for survival.

During the first ten to eleven days the degenerative process in the homoplastic transplanted grafts appears mainly as in autoplastic transplantations; that is, like a Wallerian degeneration, only a little more slowly. Myelin ovoids are formed, the cells of Schwann multiply, and immigrated phagocytic cells loaded with fatty granules are seen in the fibers (Fig. 9). From the eleventh to twelfth days, these cells are present in a number considerably exceeding those in the autoplastic grafts. They are steadily increasing in number, and from the sixteenth to eighteenth days (Fig. 10) they form a marked feature in the whole picture.

It is possible that the presence of these numerous immigrated cells in homoplastic grafts—cells provided with phagocytic properties and probably of lymphocytic origin—are playing some part in the mechanism of immunity against homoplastic transplantation of tissue in general. For in transplantation of organs and tissue there is a marked difference between the final result of homoplastic and autoplastic transplantation (the kidneys, for instance). But I do not wish to enter upon further discussion of the importance of the phagocytic cells. I wish only to add that in homoplastic nerve grafts the nuclei of Schwann from the eighteenth to twentieth days are pale and faintly stained and are evidently in a necrobiotic condition, which is possibly dependent upon the presence of the lymphocytes.

Heteroplastic Transplantations.

In heteroplastic transplanted nerves an abundant formation of myelin ovoids occurs during the first four to five days. But later these grafts do not resemble either the autoplastic or homoplastic transplanted pieces or nerves in Wallerian degeneration. There is no proliferation of the cells of Schwann. These cells, on the contrary, are faintly stained or have completely disappeared. From the eighth and tenth days the contents of the fibers consist mostly of

irregularly broken up pieces and fragments of myelin and protoplasm and the whole fiber looks necrotic (Fig. 11).

In later stages we find between and in the fibers numerous immigrated cells, and from the sixteenth to eighteenth days the graft on gross examination is yellowish, soft, and necrotic, or it is encapsulated by young connective tissue.

SUMMARY.

In autoplastic transplanted nerves a degenerative process occurs which resembles the ordinary Wallerian degeneration, but appears a little more slowly than the latter. The cells of Schwann are in a condition of survival and are capable of multiplication after the transplantation.

In homoplastic transplanted nerves I have found a degenerative process resembling a Wallerian degeneration, somewhat delayed. The cells of Schwann multiply, and for some time at least are in a condition of survival. After twelve to fourteen days an abundant and increasing immigration of lymphocytes is observed, and from the eighteenth day the cells of Schwann develop a necrobiotic appearance.

In heteroplastic transplanted nerves numerous myelin ovoids are formed during the first four to five days, but there is no proliferation of the cells of Schwann, and no Wallerian degeneration is seen. The graft becomes necrotic within about two weeks.

The formation of ovoids that occurs during the first four to five days after the performance of the heteroplastic transplantation does not reveal the condition of the life of the graft. This formation of myelin ovoids is found in the nerve fibers when they have been kept in an incubator for twenty-four hours in Ringer solution (Nageotte[12]) or in homologous or heterologous serum, but it is not found in the fibers after their incubation in isotonic salt solution (Nageotte), the presence of calcium being necessary for the occurrence of ovoid formation.

[12] Nageotte, J., *Compt. rend. Soc. de biol.*, 1910, lxix, 556.

CONCLUSIONS.

Heteroplastic transplanted nerves become necrotic. They are unsuitable for bridges in cases of nerve defects, and my results explain the failure of the attempts at heteroplastic transplantation of nerves in human beings.

If we wish to bridge a nerve defect by implantation we must use autoplastic or homoplastic grafts. The occurrence of a Wallerian degeneration in these grafts during the first two to three weeks after the transplantation should make bridging a promising operation; for in this period the grafts resemble the peripheral part of a divided nerve and must be assumed to be capable of regeneration, and thus are very different from dead material.

I have studied the process of regeneration, and shall communicate in a future article my results of bridging defects, which are encouraging as far as the function is concerned.

My results with homoplastic transplantation of nerves have a bearing on the homoplastic transplantation of limbs, which has been successfully performed in dogs by Carrel. None of his dogs lived long enough to show any function of the transplanted leg. The practical value of this operation is dependent, of course, upon the return of function, and especially on the regeneration of the nerves in the transplanted leg. The results with homoplastic transplantation of nerves seem to indicate the possibility of a regeneration of the nerves in a homoplastic transplanted leg.

EXPLANATION OF PLATES.

PLATE 44.

Figs. 1, 2, and 3 are nerve fibers from the peripheral part of divided nerves. Wallerian degeneration in different stages.

FIG. 1. Wallerian degeneration, 4th day.

FIG. 2. Wallerian degeneration, 7th day.

FIG. 3. Wallerian degeneration, 14th day. Multiplication of the nuclei of Schwann, numerous immigrated cells (*"corps granuleux"*).

FIG. 4. Nerve fibers from a graft, 6 days after transplantation (autoplastic). Multiplication of the nuclei of Schwann.

FIG. 5. Nerve fiber from a graft, 16 days after transplantation (autoplastic). Long rows of nuclei of Schwann. Some immigrated cells (*"corps granuleux"*).

PLATE 45.

FIG. 6. Nerve fiber from a graft, 18 days after transplantation (autoplastic). Multiplication of the nuclei of Schwann. Reduction of the myelin ovoids. Numerous immigrated cells.

FIG. 7. Nerve fibers from a graft, 4 days after transplantation (homoplastic). The nuclei of Schwann have fallen in towards the center of the fibers, embedded in protoplasm.

FIG. 8. Nerve fibers from a graft, 4 days after transplantation (homoplastic). In one of the fibers a mitotic figure is seen in a cell of Schwann.

FIG. 9. Nerve fibers from a graft, 10 days after transplantation (homoplastic). Multiplication of the nuclei of Schwann. Immigrated small darkly stained cells (*"corps granuleux"*).

PLATE 46.

FIG. 10. Nerve fiber from a graft, 18 days after transplantation (homoplastic). Multiplication of the nuclei of Schwann. These nuclei are pale and faintly stained. A large number of immigrated cells is seen.

FIG. 11. Nerve fibers from a graft, 10 days after transplantation (heteroplastic). No cell is seen. The fibers appear necrotic.

(Ingebrigtsen: Biology of Peripheral Nerves in Transplantation.)

FIG. 6.

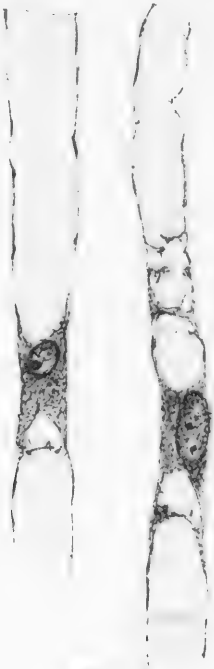

FIG. 7.

FIG. 8.

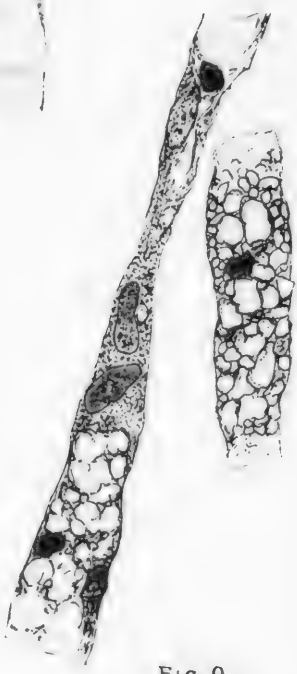

FIG. 9.

(Ingebrigtsen: Biology of Peripheral Nerves in Transplantation.)

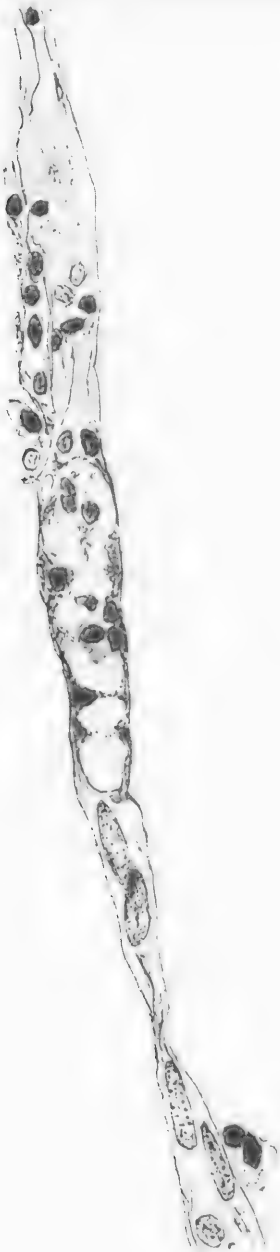

FIG. 10.

FIG. 11.

(Ingebrigtsen: Biology of Peripheral Nerves in Transplantation.)

THE DIAGNOSTIC VALUE OF THE PLACENTAL BLOOD FILM IN ÆSTIVO-AUTUMNAL MALARIA.

By H. C. CLARK, M.D.

(*From the Board of Health Laboratories, Ancon.*)

PLATE 47.

(Received for publication, May 22, 1915.)

Any additional factor that can be placed in the hands of the diagnostician to aid in the differentiation of diseases seems worthy of introduction; therefore, the examination of the placental blood film is recommended to indicate better the presence of æstivo-autumnal malaria when it complicates labor and in differentiating it in the early days of the puerperium from puerperal sepsis.

The cause of a fever in the puerperium is not very difficult to find when the case is under competent observation in a non-malarial region, but the diagnostic significance of such a fever in the tropics may require further analysis to eliminate latent or active protozoal disease.

Malaria, as a cause of death in the Panama Canal Zone, ranks well below several other factors, yet it has been a very important one in morbidity statistics, and all divisions of the hospital service at Ancon have been obliged to keep it in mind as the single or a possible associated factor in seeking to establish a diagnosis.

Marchiafava and Bignami (1) feel that the diagnosis of malaria can be made without a puncture of the spleen if examinations of the peripheral blood are made often enough and with sufficient care. This statement has been largely confirmed by James (2) in his experience with malaria on the Canal Zone, in so far as it relates to individuals who are incapacitated by this disease and in many cases where it seemed to be merely a coincident factor; but there are examples of a latent type, or individuals who may be called malarial carriers, that will not reveal the presence of the parasite in a single examination of the peripheral blood and sometimes even over a long

period of time unless some factor of an intercurrent nature lowers that individual's resistance and encourages a malarial outburst.

It occasionally happens that the physician suddenly assumes the responsibility of a case in labor associated with a severe attack of malaria which is at the moment between the periods of sporulation; in such instances the examination of the placental blood film will expedite the diagnosis and permit the immediate institution of treatment.

Puerperal fever in regions where æstivo-autumnal malaria is endemic always offers some reason for anxiety to the conservative practitioner who wishes at the earliest moment to differentiate malaria and puerperal sepsis; while to the other type malaria affords one of the safe retreats in diagnosis to cover all fevers which appear in the puerperium.

The evil relation of malaria to the course of pregnancy and the puerperium has long been given consideration by various writers on tropical medicine, but the primary intention of this paper is to advocate the routine examination of the stained fresh placental blood film as an immediate means of identifying the presence of malaria in nearly every case when it is associated with labor or the early days of the puerperium.

It has long been known that it is possible to find an abundance of parasites in a blood film made from the maternal surface of the placenta in a woman whose labor was complicated by a serious attack of malaria, but as far as I can learn no practical application of this fact has ever been made, it being simply considered a curious feature sometimes encountered.

I believe that the Ancon clinical and pathological data soon to be mentioned, together with the results of 400 observations on placental blood films, will show that it has a practical application and is a worthy test for routine practice in tropical obstetrics and in other regions where this type of malaria is found. This is the only instance in which the body voluntarily offers for examination an intricate capillary or blood sinus system that closely approximates such vascular systems as the spleen, bone marrow, and liver, and the fact is well established that the adult forms of the æstivo-autumnal parasite remain, as a rule, stationary in such systems.

Unfortunately the value of this feature did not impress itself at a time in the course of the construction of the Panama Canal when it could have been applied to the largest number of individuals who were the most susceptible to malaria, and also before the sanitary system was operating so successfully against this disease.

However, the study of this short series of 400 cases, made under the present adverse circumstances, may yet reveal sufficient evidence to impress the importance of such an examination, and it will at the same time offer other points of interest.

Pathological Observations.

Some time ago a collection was made, for general study and analysis, of all fatal cases of æstivo-autumnal malaria that had come into my hands through the routine pathological service of the Board of Health Laboratories during a period of about four years. There were included in this series of 107 cases 31 females of whom 7 were children. Among the remaining 24 adult women, 12 were found to have been either in the late stages of pregnancy or not far advanced in the puerperium. This fact seemed so significant that the general anatomical records covering the same period of time were again searched to see what further relation could be found between other conditions and the pregnant state.

192 autopsies were found that had been performed on adult females. 26 of this number were either pregnant or had been recently delivered. The following relationship was found:

Malaria, æstivo-autumnal	12	cases.
Malarial hemoglobinuric fever	3	"
Puerperal septicemia	4	"
Eclampsia	3	"
Typhoid fever	2	"
Chronic nephritis	1	"
Extra-uterine pregnancy	1	"

The race of the cases showing an association of the pregnant state with æstivo-autumnal malaria and malarial hemoglobinuric fever is shown in Table I.

TABLE I.

Race.	No. of cases of malaria.	No. of cases of malarial hemoglobinuric fever.
Negroes, West Indian	8	1
White, Spain	2	2
Mestizo, Panama-Colombia	1	0
White, United States; never under medical care	1	0

Clinical Observations.

Through the kindness of Dr. A. B. Herrick, Chief of the Surgical and Obstetrical Clinics, and of Dr. W. E. Deeks, Chief of the Medical Clinic, their Ancon Hospital Records covering the same period of time as the pathological records just mentioned were next appealed to along the same lines, and after excluding those cases contained in the anatomical report the following additional data were assembled (Table II).

TABLE II.

Race.	No. of cases of miscarriages and abortions.	No. of cases of normal pregnancy.	No. of other accidents of pregnancy and labor.	Total.	No. of positive malarial cases.
Americans	85	340	12	437	1
West Indian negroes	54	295	36	385	31
Europeans	9	66	1	76	2
Latin-Americans	14	102	6	122	3
Chinese	0	2	0	2	1
Syrians	0	1	0	1	0
Total	162	806	55	1,023	38
No. of associated malarial attacks.	14	16	8		38

Among the 38 cases there were 5 deaths not included in the list of autopsies.

It is not possible to confirm entirely by these statistics the more or less general opinion that in the tropics where malaria is endemic the great increase in abortions and miscarriages is chiefly due to malaria. There are too many other factors which, no doubt, play a part in this and have not been ruled out by the authors of such an opinion. However, there is present in the data a very striking relationship between many of these accidents and associated malaria as compared to other diseases such as pneumonia, typhoid, tuberculosis, and even syphilis.

Tuberculosis leads all other factors as a cause of death in the

Canal Zone during this period of time, in so far as a record of autopsies can be taken for a guide. Pneumonia also stands well ahead of malaria as a cause of death. Syphilis is a common disease on the Zone in these days, but armed with a clinical test it can be well identified; yet the combined anatomical statistics on the known evil association of other diseases with pregnancy and the puerperium do not exceed the results which malaria alone has accomplished, and this fact is well supported by the clinical data.

The various opinions expressed by Scheube (3), Deaderick (4), Williams (5), Craig (6), and others led to the taking of further observations before expressing any view based on the local data just mentioned.

Conditions have rapidly changed on the Canal Zone since the clinical and pathological reviews were made, there being a marked reduction of people in the Canal Zone and also of the malarial rate. Many villages have been abandoned from which such cases were received. In addition to these factors the new observations have been conducted chiefly in the dry season. Nevertheless, the examination of 400 cases of labor has been made on individuals from various parts of the Canal Zone, the period of time beginning August 21, 1914, and ending March 30, 1915.

Methods Employed in Making the Observations.

At the end of labor some blood films were made from the mother's peripheral blood by the physician in charge of the case, and these films and a history of the case together with the carefully protected placenta and umbilical cord were sent to the Board of Health Laboratories at Ancon, where an immediate opportunity was given me to prepare the placental and fetal blood films.

Great care must be taken in preparing the fetal blood film from the blood in the umbilical cord to prevent contamination of this specimen with the placental blood. False impressions of the infected fetal blood can easily be obtained unless precautions are taken.

The maternal surface of the placenta when it was received at the Laboratory was usually covered over with the membranes, and the folds of these membranes often contained a large amount of maternal blood. It has, therefore, been the custom to prepare first the film from the umbilical cord. The cord is thoroughly cleaned and

then cut near its middle, and the oozing blood caught and spread in the usual manner. This film is marked and laid aside before disturbing the placenta and membranes.

The placental blood film is made from the maternal surface of the placenta after all free blood and clots have been removed from its surface and from the folds in the membranes. Blood was made to ooze from the clean surface by grasping a portion of it with a hemostat. The specimen was then removed by pressing down and dragging the end of a glass slide across the bleeding surface, and from this material the films were made. The three types of blood films were then dried and stained with a polychrome stain (Hastings) in the usual way that the ordinary blood film is treated. Then followed a routine examination for the presence of the malaria plasmodium.

The Wassermann test was applied to many of the cases and invariably to all suspicious cases whenever the state of the blood contained in the fetal membranes was such that it could be used for the test. If a dead fetus accompanied the placenta and cord and its condition permitted the application of the test, it was performed. Suspicious lesions in the placenta or in a dead fetus were prepared by the Levaditi method to permit a search for *Treponema pallidum*. This organism was also kept in mind when the blood films were searched. The series was closed after the receipt of 400 specimens that were in a suitable condition for examination.

The results are presented in Tables III and IV.

TABLE III.

400 cases examined.	No. of positive cases of malaria.
Blood films from maternal surface of placenta	19
Blood films from mother's peripheral blood	8
Blood films from umbilical cord	1

400 Routine Cases of Labor.

Race of the women examined.	No. examined of each race.	No. of positive identifications of malaria.	Per cent of positive cases.
North Americans (white)	118	0	0.0
Latin-Americans (mestizo)	92	3	3.26+
Europeans (white)	17	1	5.88+
West Indian negroes	173	15	8.67+
Total	400	19	4.75

TABLE III.—*Concluded.*

Local Residence of the Positive Cases.

Residence.	No.			
Unknown suburban division of Panama City	4	Panama City	15	Colon
Calidonia	4			
Guachapali	3			
San Miguel	2			
Maranon	1			
Chorrillo	1			
Mount Hope	1	Colon	3	
Colon	2			
Culebra	1	C. Z.	1	
		Total	19	

Seasonal Incidence of the Positive Cases.

August	0	December	3
September	2	January	2
October	4	February	1
November	3	March	4

Labor Accidents That Were Known To Have Occurred in the Series.

Abortion	9
Premature labor (fetus dead in 13 of these cases)	23
Full term still-births	11
Full term children dying inside of 48 hrs.	1

A total of 44 accidents in the series of 400 cases.

There were 19 positive cases of æstivo-autumnal malaria in the 400 cases, and 7 of these 19 cases (a percentage of 36.84+) were found associated with some of the accidents named above.

Besides the 19 cases positively identified as malaria, there were 5 other cases with a reliable history of having been treated for malaria in recent months, and in 2 of these, full term still-births were recorded; while in a 3d case a grave anemia and premature labor were noted. Thus, if 24 be accepted as the actual number of cases with a malarial influence present, and 10 the number of accidents, a percentage of 41.66 is revealed. Other factors found in connection with some of these accidents where no malaria influence was established are given in Table IV.

TABLE IV.

A calculus in the left ureter and left pyonephrosis	1
Grave anemia	1
Acute endometritis, suppurative	3
Eclampsia	1
Typhoid	1

27 of the accidents thus remain in which no plausible factor at the time of labor could be named as the cause.

The racial classification of the women in which the total number of accidents occurred is as follows: Americans, 14; Europeans, 6; Latin-Americans, 6; West Indian negroes, 18.

Discussion of Tabulated Features.

The placental films revealed the presence of malaria in eleven cases whose peripheral blood appeared negative. On the other hand, the peripheral blood was never found positive without the placental film showing a much heavier infection. This is an important difference and one which might be expected when the general circumstances, surrounding the origin of the two films, are given consideration. The adult parasites, especially of the æstivo-autumnal type, are prone to remain stationary in the more complex capillary systems of the deep viscera, and after the third (7) or fourth month of its development the placenta belongs to that class of viscera. It rapidly increases in importance as a favorable site for the development of adult parasites as its maturity is reached. In most instances the first field examined in the placental films will be sufficient to establish the diagnosis of malaria in untreated cases, even though a tedious search may have been required to locate parasites in the peripheral blood of the same case.

The malarial carriers identified in this series by examination of the placental film and not found positive in their peripheral blood required not more than one to five minutes in locating the first parasites. Seldom will it be necessary to make a protracted search of this film if malaria is present during labor and quinine has not been previously administered.

In some of the cases identified in this group the infections were so heavy that a fatal issue seemed certain for both the mother and child, yet in all instances the mother lived and in nearly all cases the child lived. The vitality of some of these jaundiced, premature children is remarkable. A few of the patients among the positive cases had been admitted just before or at the time of labor and were apparently between the periods of sporulation, since there was no temperature, yet heavy infections were noted in the placental films. Even with this knowledge of these cases in mind, a tedious search of the peripheral blood was required to find the ring forms. When under short observation these cases can occasion unpleasant surprises.

The average numerical relationship between the parasites found during a clinical attack of malaria in these three types of films can

be more easily appreciated by inspection of Figs. 1 and 2 which have been drawn from the actual preparations made in such a case. Fig. 2 representing the placental film contains parasites drawn from three cases in order to present some common features of interest that will be explained in the key to the illustrations. The numerical relationship there represented is, however, accurate for the conditions named.

The commonest types encountered in the placenta are the segmenting and presegmenting forms. It also frequently happens that the majority present are younger adult forms. Crescents were seldom found, yet they are common forms in the films made from bone marrow and the spleen. Sometimes the large forms seem entirely free from red blood cells, but expert variation in the method of staining might have shown blood cell remnants.

The phagocytosis of pigment in the placenta is not so extensive as it is in the spleen, liver, and bone marrow, but it is quite marked and often even the polymorphonuclear leucocytes take part in this activity.

It is a much easier task to prepare and examine a placental film at the time of labor than to spend perhaps long periods of time on each of two or three consecutive days following labor in an effort to catch the products of a sporulation in the peripheral blood of the patient; because, in the placenta, the parasites are quite large and very abundant and there is much evidence of phagocytosis, while in the peripheral blood a few small ring forms are all that can usually be expected.

The study of the fetal blood from the umbilical cord presents a feature of interest in regard to the evidence it affords in support of the view that the mother's blood and the fetal blood never intermingle under normal conditions.

A single instance occurred in this series where an undoubted moderate infection of the fetal blood existed at the time of birth. The mother's blood and the placenta bore evidence of a very heavy infection. There was the history and the evidence of an associated accident of pregnancy in relation to the placenta so that it seems wise to attribute this infection to the placental accident. The mother and child were discharged in such a short time from the institution in which the case occurred that no further observations

on the child's blood could be taken. The final result is not known, but the mother was a black woman and the probabilities are strong that both mother and child are well. This is the only case in the last five years that has come under my observation that can certainly be established as an intra-uterine transmission of malaria to the fetus. Our few cases of malaria in infants are all surrounded with so many circumstances favorable to postnatal infection that they cannot be safely considered cases of congenital malaria. As a matter of fact it seems reasonable to assume that the observations made in this series alone offer good support to the long standing belief that the blood streams of the mother and the fetus never actually intermingle unless some rare accident to the placenta occurs.

It seems necessary to impress again the fact that three important features existed during the collection of these cases which have greatly lessened the number of infections otherwise to be expected in such a number of individuals. The sanitary system continues with ever increasing success to exert a strong influence against this disease. The reduction in the population of the Canal Zone has been great. The observations have also been carried on partly in the dry season, at which time this disease is supposed to be at a low ebb.

White women coming from the temperate zone of the United States and Europe, who live on the Canal Zone, are believed to be the most susceptible women to malaria, but nearly all European women have gone and the Americans avail themselves of all the opportunities offered by the system of sanitation as well as professional care throughout their course of pregnancy.

This leaves only the negro women, whose race seems to enjoy a relative immunity to malaria, and the native women, who possess even a higher grade of immunity than the West Indian negro. Under the circumstances, however, it does not seem unusual for the highest rate of infection to be found in this series in the negro. The negress is the domestic servant and she lives under limited circumstances chiefly in the suburban divisions of Panama City where the rent is lowest and the malarial rate highest.

These conditions no doubt explain the high rate in the relatively immune negress and the absence of the disease in the non-immune

white women. The same rate and character of infections in the white woman that have been found in the black woman in this instance would certainly have been accompanied with more serious consequences.

The negress is very indifferent to her pregnant state and seldom can tell within a month or two the correct time for her course of pregnancy to end. It is therefore quite possible, judging from the appearance of some of the children, that several more premature labors associated with the malarial cases in this series should be recorded; but the histories offered in these instances have been adhered to and they were not counted among the accidents.

It seems incredible that a fetus can live where nutrition is derived from such heavily infected placental blood, yet in a remarkable number of cases (negroes at least) they do survive. In some cases this may be due in part to the fact that latent malaria many times does not show a terrific outburst until very late in pregnancy when the patient's vitality is low and the placenta a better harbor to assist in the development of parasites. At this time the child may be sufficiently developed to fight for an independent existence forced upon it by miscarriage. The probabilities are that its chance for life is better under even these conditions than to continue its intrauterine existence during the climax of a severe malarial infection.

The placenta is one malarial battle-field over which the body holds the ruling influence, for by expulsion of the uterine contents the field and its contained enemies are quickly eliminated.

The later influences of such a birth as this on the infant can only be a subject for conjecture. It would certainly appear to offer a predisposing cause to the diseases of early infancy. When a fetus is developed throughout its uterine life in association with latent malaria in the mother and at least in contact with one acute attack in the mother it would seem that there should be some degree of immunity gained to this disease by the child in after life even though the parasites were never admitted to its circulation from that of the mother. This thought recalls an opportunity afforded me a few years ago to study microscopically the tissues of a still-birth associated with fatal malaria in the mother. The tissues happened in this instance to be in good condition. Its blood films and those

made from its spleen were negative for parasites, yet when the histological study was made, a condition comparable to the cloudy swelling associated with deaths due to febrile diseases and abundant microscopic evidence of pigment was found that I am not able to tell from the melanin of malaria. It is difficult to say whether this pathology was due to toxins in the mother's blood stream or simply to interference with the nutrition of the fetus. The deposition of the pigment in its tissues is also open to several lines of thought.

GENERAL DISCUSSION.

Scheube (3) states that disorders of menstruation and a decrease in fertility are more frequent in European women, and miscarriages also occur more often with them in the tropics than when they live in Europe, and he adds the further statement that climate is less to blame for this than malaria. This opinion is given more or less general support and there is no doubt that malaria does bear a very important relation to these conditions, yet there are many other factors in the tropics where malaria is endemic that help to make these features more common.

Williams (5) offers a counter-statement: Despite the somewhat wide-spread opinions to the contrary, it would appear that ordinary forms of malaria have but little influence upon the course of pregnancy; it is probable, however, that the pernicious forms of malaria may have a much more deleterious effect. He admits the tendency to malarial outbreaks in pregnancy and the puerperium.

A compilation of the various records in regard to the malarial influence in pregnancy and an additional valuable opinion is found in Deaderick's (4) writings.

The experience at Ancon indicates that æstivo-autumnal malaria does exert frequently an evil influence in the late stages of pregnancy and in the puerperium, sometimes being fatal to both mother and child, but there yet remains a very large number of miscarriages, abortions, instances of sterility, and menstrual disturbances that cannot be explained, and least of all can they be ascribed to malaria on any other than circumstantial data of a high rate of malarial morbidity in the tropics.

The anatomical structure of the placenta in the late stages of

pregnancy and the opportunity it offers for the development of the malarial parasite makes it a point of strategic importance for the parasite in interrupting the late course of pregnancy, and it is surprising that it does not always do so. What harm may come to the menstrual process and to fertility in untreated malarial carriers who are apt to suffer from numerous small outbursts of malaria is less easy to answer. Such a circumstance would probably play an important part.

The results and statements of the physicians in charge of obstetrical work at Ancon Hospital offer confirmation to Williams' (5) statement that quinine can be given with impunity in such conditions. The accidents number about the same with or without its administration, and certainly the mother's prognosis is better when it is given.

It is hard to dismiss the idea that children born under such conditions, if they survive, do not gain at least a temporary immunity from the disease. It is extremely rare to receive at the morgue in Ancon a child under one or two years of age dying of malaria. Observations have been made, however, by competent people on large amounts of clinical material who claim that all malarial immunity is acquired.

These children, born of mothers with a positive malarial infection, will be followed, as far as possible, to determine their future relation to the disease and to see whether their birth under such conditions plays a predisposing part in making them more easily the victims of other diseases of infancy.

The observations of Bastianelli and Bignami (8) on the punctiform hemorrhages, which sometimes occur about the vessels in the brain in some of the severe cases of æstivo-autumnal malaria, support the theory of a viscid property possessed by this parasite, and Bass (9) has offered further proof of this fact. These small punctate hemorrhages were said to consist of uninfected red blood cells, while the cells, visible in the capillaries about which the hemorrhages were found, were all infected cells. An explanation offered is that the infected cell anchors itself to the endothelium of the vessel, while flexible non-infected cells under pressure escape by diapedesis. I have personally observed instances of these hemorrhages and can

confirm their statement in regard to their composition and the nature of the cells in the capillaries near them.

A somewhat parallel circumstance occurred in another instance in which a fatal termination of a severe case of æstivo-autumnal malaria was brought about by the spontaneous rupture of the spleen and hemorrhage into the peritoneal cavity. The free blood in the peritoneal cavity unexpectedly revealed the discoid and ring forms commonly found in the peripheral blood, while films from the splenic pulp revealed an abundance of the larger adult forms. This circumstance, I believe, also supports the theory that these larger parasites or the infected cells possess a greater degree of viscidity and can maintain their position in the favorable locations mentioned. The higher degree of body temperature probably assists in the development.

Labor, associated with malaria, spontaneously offers the morphologist an opportunity to study at the same moment the parasites of the peripheral and deep circulation. Even at the end of twenty-four hours a heavily infected placenta can often be subjected to an examination with good results, but this necessitates a knowledge of the degenerated forms of the parasite and a more intense method of staining.

SUMMARY.

1. The placental blood film examination is worthy of routine application wherever æstivo-autumnal malaria is endemic. This type of malaria when associated with labor and the early days of the puerperium can be more easily and certainly diagnosed by the use of this film and a polychrome stain than by employing the usual films made from the mother's peripheral blood at the time of labor.

The placental film in such an infection offers an abundance of adult parasites and far more evidence of the presence of pigment, while the peripheral blood film frequently offers but a scant number of the small ring or discoid forms of a parasite. The examination of the present series revealed positive placental films in nineteen cases, while but eight of these same cases were positive in the peripheral blood film examination. On the other hand, no peripheral blood films were found positive in which the associated placental films did not reveal a far more abundant evidence of the infection.

2. The early days of the puerperium can by this method be protected many times from a malarial outburst, and, as a rule, puerperal sepsis can be differentiated.

3. The intricate vascular architecture of the mature placenta rivals that of the spleen, liver, and bone marrow as a harbor for adult malarial parasites of this type and as a storage for pigment.

4. The localization of parasites in the placenta is unique. Here is the one vascular system which particularly favors the development of the parasites but which at the same time is so situated that it may be spontaneously discarded by the body at the climax of the attack. By this simple act late in pregnancy the prognosis for both mother and child may be improved.

5. The pregnant state encourages attacks of malaria by lowering bodily resistance and by furnishing an additional harbor for the development of parasites. A tenable theory in regard to most attacks of this nature, occurring in cases under professional care, would appear to be the development of latent malaria (malarial carriers) into acute attacks toward the close of the pregnant state. The women who expose themselves (as the negroes in this series) offer favorable conditions to the introduction of a primary infection.

Malaria frequently interrupts the late stages of pregnancy and sometimes causes the death of the mother and the fetus, more often the latter. The records at Ancon indicate that it more frequently exerts a harmful influence than other types of infectious diseases in this locality.

6. Most of the children in this series that were delivered while malaria was present in the mother, were of a race that seems to possess a relative immunity to the ravages of malaria. This may account for the fact that the negro fetus more nearly approximates the full term of development when associated with this disease and is comparatively subjected to a less number of the accidents of pregnancy. Many of them revealed evidence of prematurity and were jaundiced, but, as a rule, they developed rapidly.

The commonest mishap is miscarriage late in pregnancy. Occasional still-births occur and sometimes there is a fatal issue to both the mother and child.

7. Cases diagnosed as congenital malaria probably indicate that

some accident occurred to the placenta, because it practically never happens that fetal blood is positive at the time of birth, regardless of the degree of infection in the mother.

Many of the cases now reported in the literature as congenital malaria suggest immediate postnatal infection as their history, as our pathological and clinical records testify.

8. The size of the intervillous spaces of the placenta and their adaptability in the localization of parasites seem to disprove to a certain extent the old idea that the localization depends on the smallness of the capillary caliber. If this were the case the brain should be more often the seat of an extensive localization than the spleen, bone marrow, and placenta, yet our anatomical records will not support that theory. A sluggish blood sinus with a large endothelial surface, a higher internal body temperature, and red blood cells burdened with parasites of a certain age beyond the ring form seem to be important factors in the localization and development of the æstivo-autumnal parasite.

9. The racial disparity of malarial infections shown in this series is believed to be due to local conditions and a wrong impression is apt to be given by our statistics in regard to the relative immunity of the negro race.

The white women on the Canal Zone avail themselves of all the opportunities the sanitary system affords; they live well and place the entire course of their pregnant state under competent professional care, while the negro woman is indifferent to her pregnant state, works as a domestic servant, and lives in the cheapest unprotected quarters that can be rented in the suburban divisions of Panama City where the malarial rate is highest and the sanitary control is difficult.

It should be noted that these negro women can carry an infection with little manifestation of its presence that would produce serious results in the white women brought from the temperate zone regions of Europe and the United States.

Thanks are due Dr. Samuel T. Darling for help rendered in preparing the paper, and to various members of the sanitary staff who have made it possible to assemble the material for study.

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EXPLANATION OF PLATE 47.

The comparative appearance of the types of films used in the study of the 400 cases are here represented by Mr. George Newbold. The primary intention of the drawings is to show the numerical relation of the parasites in each type of the films. The case selected for the drawings was one in which the individual on admission to the hospital gave no evidence of the infection, but soon after labor showed a moderate rise in temperature. Fig. 1 was drawn true to the actual conditions found in the films, but Fig. 2 was made into a composite drawing of parasites found in three separate cases, in order to represent some common phases which occur in this type of film. However, the figures are true to the average numerical relationship found in a simple attack of æstivo-autumnal malaria complicating labor.

Fig. 1. A film from the mother's peripheral blood stream stained with Hastings' polychrome modification. This film revealed an average of about 3 parasites to each microscopic field examined (obj. 1/12, oc. No. 8).

A, B, and C represent three forms of the young parasite.

D represents a large lymphocyte with a clump of phagocyted pigment.

E is a polymorphonuclear leucocyte.

Fig. 2. A composite drawing made from the placental blood films of 3 cases. Two of the cases show phases of degeneration of the parasites due to the interval of time which passed before films could be prepared from the specimens received.

A is a large lymphocyte showing phagocytic activity. These cells are more commonly observed here than in the peripheral blood.

B and C represent the commonest types of parasites encountered in the fresh placental film, segmenting and presegmenting forms. Many times the field will be crowded with these forms, while in lighter infections 1 to 5 parasites to the field are found, and in these instances it seldom happens that any parasites can be found in a peripheral blood film.

D represents the appearance of such parasites as C when 24 hours have elapsed following labor, before the film could be prepared. By the use of expert methods of counterstaining the Hastings' preparation with Giemsa stain, these parasites can be made to resemble presegmenting types.

E is the smallest parasite usually found in this film.

The remaining parasites, which show poor definition and stain deeply and irregularly, represent films made from a case 10 or 12 hours after the end of labor. Magnification the same as in Fig. 1.

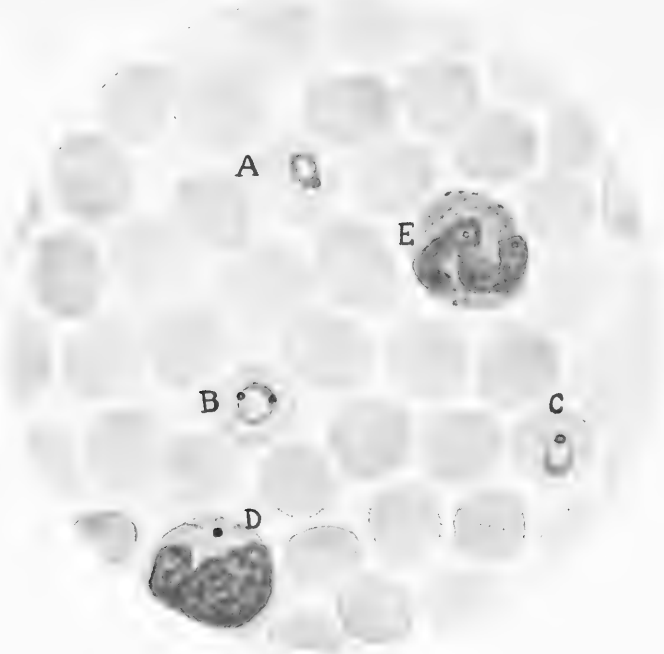

FIG. 1.

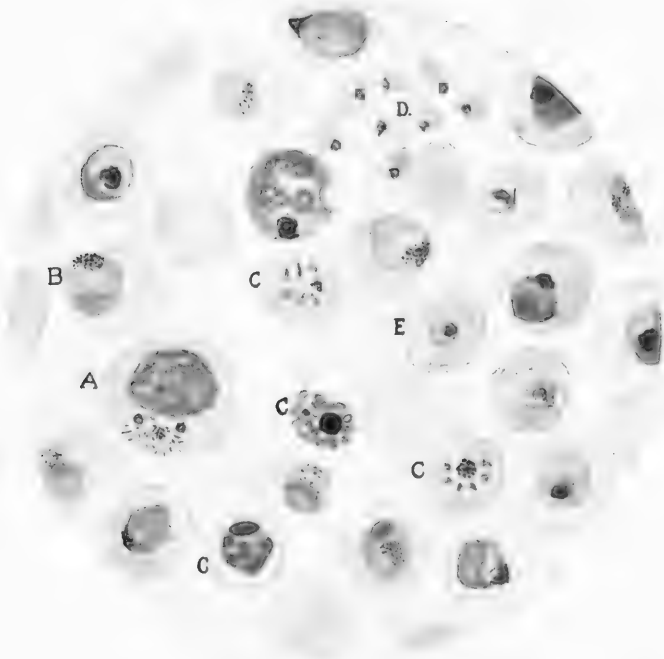

FIG. 2.

(Clark: Value of Placental Blood Film in Malaria.)

AN IMMUNOLOGICAL STUDY OF BACILLUS INFLUENZÆ.

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Plate 48.

(Received for publication, June 15, 1915.)

Bacillus influenzæ is a frequent invader of the human body, where it either causes or complicates important pathological processes. Among the pathological conditions which it produces are bronchopneumonia, empyema, and leptomeningitis; and among those in which it accompanies streptoccoci and pneumococci are the common laryngeal, tracheal, and bronchial affections. Moreover, it is sometimes associated with these cocci in lobular and lobar pneumonia.

According to the location and severity of the infections the influenza bacilli remain confined to the local lesions, or at the same time invade the blood stream. When they are confined to the local lesions in the respiratory organs, the bacilli tend to be of low virulence for animals. When they invade the blood, as they do in leptomeningitis and in some instances of pneumonia, they tend to be of higher virulence. Along with the blood invasion, influenzal suppurative arthritis may occur. These facts suggested the problem as to whether the strains of influenza bacilli isolated from various sources are identical, or whether they may be differentiated into groups on sound biological or serological grounds.

Sources of the Cultures.—The strains of *B. influenzæ* employed in this study came from two main sources; first, the respiratory mucous membrane or the lungs; second, the cerebrospinal fluid. The respiratory strains were usually slightly virulent, the other strains more virulent for laboratory animals. The bacilli isolated from the blood both before and after death were always compared with the respiratory and meningeal cultures obtained during life from the same case. This applies also to the strains isolated from

suppurating joints occurring as a complication of influenzal bacteriemia with meningitis.

Criteria of Virulence for Animals.

The subject of virulence or pathogenicity of *B. influenzæ* for animals has been dealt with in previous papers.[1] It will suffice to recapitulate the main facts in this place. The white mouse succumbs to intraperitoneal injections of cultures irrespective of their origin. A peritoneal exudate arises which contains large numbers of the influenza bacilli, as does the heart's blood. Guinea pigs of 200 grams' weight, on the other hand, succumbed to the intraperitoneal injection of one blood agar culture of all the meningeal, and to about one-half of the respiratory, strains tested.

It is by means of the rabbit that the distinction of the virulent or pathogenic strains from those lacking this quality is accomplished. Rabbits of about 1,000 grams' weight are employed. Using one blood agar culture injected intravenously as the test dose, nineteen of the twenty meningeal strains tested caused death within eighteen to thirty hours. The heart's blood in fatal cases contained large numbers of the bacilli. The respiratory strains are for the most part non-pathogenic for rabbits. Among several score of such strains I encountered only six which were virulent for these animals. Four of them came from the lungs and heart's blood and two from the lungs alone (the heart's blood being free) of infants at the Babies' Hospital. In none of the six cases were the leptomeninges involved. In this connection it may be mentioned that Batten[2] described a strain of *B. influenzæ* obtained from the meninges which was wholly devoid of pathogenic properties for mice, guinea pigs, and rabbits.

Morphology.

B. influenzæ is subject to marked variations in morphology. Two main forms are met with, the short and the long. The respiratory strains usually belong to the first class. In films from the bronchial secretion influenza bacilli are short and rather thick, but regular in

[1] Wollstein, M., *Am. Jour. Dis. Child.*, 1911, i, 42; *Jour. Exper. Med.*, 1911, xiv, 73.

[2] Batten, F. E., *Lancet*, 1910, i, 1677.

size. On blood agar plates dew-drop colonies are developed which can be readily differentiated from colonies of all other bacteria by their translucent, colorless appearance. The edges are quite regular, they always remain small, and they never cause hemolysis in the surrounding medium. The bacilli in such colonies are very short and regular, often coccoid in size, with deeply staining poles. No threads are formed (Fig. 4). On blood agar slants the growth is very profuse, but the small translucent colonies do not coalesce. In the condensation water of the tubes threads are formed, but they are never long. The meningeal strains belong to the second class. The bacilli in the cerebrospinal fluid are sometimes remarkably long and thick, and so little do they resemble the usual forms of *B. influenzæ* that the diagnosis of influenzal meningitis from films alone becomes difficult (Fig. 1). In other cases short, almost round forms appear in the films so that the presence of a coccus may be suspected (Fig. 2). Grown on moist blood agar plates dew-drop colonies are produced, in which the individuals may be swollen and atypical (Fig. 3). When transplanted to blood agar in which there is less free fluid, the forms become typical. Similarly, an excess of bronchial secretion tends to cause swelling of the bacilli in respiratory strains, but the typical form appears in subcultures. The matter of moisture present affects the morphology of both types. Even so the meningeal strains tend toward larger, more definitely bacillary forms, and incline to the formation of larger threads. When the meningeal strains are recovered from the peritoneal cavity of inoculated animals they are always small and more regular. But the next subculture again shows the larger bacillary forms.

The form tends to be constant for each strain. Thus, a coccoid strain is apt to remain small and regular and to produce only short threads. The bacillary forms, on the other hand, tend to long thread formation. However, after long periods of artificial cultivation (two years or more) both the coccoid and bacillary forms, as they existed, on the one hand, in respiratory and, on the other, in meningeal lesions, grow larger and acquire the power to give rise to long threads (Figs. 5 and 7). If the later generations are examined within the first twenty-four hours of growth some minute bacilli will be detected, indicating the original type to which the strain belongs.

A medium ill suited to growth leads to greater irregularity of form. Thus plain agar tubes upon which a small amount of human blood was placed gave rise upon inoculation of various strains to typical bacilli on the one hand, and, on the other, to long interlacing threads, recalling the leptothrix threads described by Ritchie[3] (Fig. 8). The latter were produced by one meningeal and several respiratory strains. Subcultures in the usual medium from these specimens yielded the typical bacilli. While meningeal and respiratory strains of *B. influenzæ* differ morphologically they present no points of difference in their method of growth on blood agar plates and slants.

Serological Reactions.

The main purpose of this investigation was, as stated, a minute study of the immunological or serum reactions of a considerable number of strains of *B. influenzæ,* with the object of determining whether the strains compose one or more groups, irrespective of virulence. For this purpose immune sera were employed, of which the decisive ones were those prepared in the rabbit with selected strains of the cultures.

Monovalent sera were obtained by immunizing rabbits to virulent and non-virulent strains of *B. influenzæ.* The virulent strains isolated from cases of influenzal meningitis were well borne by the animals in increasing doses over a period of three to five months. The respiratory strains, on the other hand, were badly borne, the rabbits becoming emaciated and dying after a dose which animals inoculated with virulent cultures were well able to bear. From these results it is fair to argue that the non-virulent respiratory strains do not produce immune bodies in sufficient quantities to protect rabbits against repeated and increasing doses of the bacilli.

Opsonins.—The opsonic content of the monovalent sera was fairly high, phagocytosis of the organisms being present in dilutions of 1 to 1,000. No specific reaction was obtained, however, the heterologous strains being taken up by the leucocytes in dilutions as high as those in which homologous strains were phagocyted.

Agglutinins.—The monovalent rabbit sera did not agglutinate

[3] Ritchie, J., *Jour. Path. and Bacteriol.,* 1910, xiv, 615.

homologous strains *in vitro* in higher dilutions than they agglutinated heterologous strains, and no reaction was obtained above a dilution of 1 to 100.

Bull's[4] experiments have shown that agglutination *in vitro* may give different results from agglutination of the same bacteria *in vivo*. Therefore, his method was used to compare the strains of influenza bacilli. Two young rabbits were inoculated intravenously with a non-virulent respiratory and a virulent meningeal culture, respectively. Blood was taken from the heart at intervals of thirty seconds to eighteen minutes, and slides were prepared. The respiratory culture showed marked clumping within one minute after inoculation, and in five minutes no bacilli could be demonstrated in the blood. The meningeal culture was not agglutinated at all by the blood of the inoculated rabbit, and at the end of eighteen minutes the bacilli were as numerous as they had been half a minute after the injection. The difference in the two results was very striking and clean cut. On killing the animals the bacilli of the respiratory strain were found within leucocytes in the liver, spleen, and lungs, while the virulent (meningeal) bacilli were found only in small numbers in the organs and were extracellular. A third rabbit was inoculated with a meningeal culture which had been isolated two years before, and which had lost its virulence for rabbits. The result was identical with that obtained with the non-virulent respiratory strain, proving again that the difference between the two strains is not absolute but only relative.

Complement Deviation.—Antigens were prepared from seven non-virulent respiratory strains and from seven virulent meningitis strains, according to the method used in the research laboratories of the New York Board of Health. I am indebted to Miss Olmstead for a description of this method, which is similar to the one described by Schwartz and McNeil[5] for gonococcus antigens, except that the suspensions of influenza bacilli are kept at 55° C. over night, since they undergo autolysis very slowly. At the end of the period of autolysis, which lasted from fourteen to eighteen hours, a differ-

[4] Bull, C. G., *Jour. Exper. Med.*, 1915, xxii, 484.

[5] Schwartz, H. J., and McNeil, A., *Am. Jour. Med. Sc.*, 1912, cxliv, 815.

ential point between respiratory and meningeal strains of influenza bacilli was noted in the appearance of the fluid. The suspensions of the meningeal cultures were invariably turbid, with a comparatively small precipitate in the tube. The suspension of the respiratory strains, on the other hand, showed a perfectly clear fluid above a large amount of precipitate. In other words, the bodies of the nonpathogenic bacilli underwent less perfect dissolution than did the bodies of the pathogenic strains. The appearances of the precipitates as revealed by stained films under the microscope were quite similar. The bacilli no longer stained deeply and were more or less disintegrated.

All the antigens were tested against two monovalent rabbit sera immune to the meningeal strains, and against two rabbit sera immune to the respiratory strains. The results are given in Tables I to IV.

TABLE I.

Complement Deviation.

Immune Serum of Respiratory Strains + Antigens of Respiratory Strains.

Complement. Guinea pig serum 1:40 dilution.	Antigen.		Immune serum (Robinson).	Anti-sheep rabbit serum 1:1,000 dilution.	Sheep corpuscles 1:20 dilution.	Result.
cc.		*cc.*	*cc.*	*cc.*	*cc.*	
0.1	1. R.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Some hemolysis.
0.1		0.1	0.1	0.25	0.25	Hemolysis.
0.1		0.2	0.05	0.25	0.25	"
0.1	2. S.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	" "
0.1		0.1	0.1	0.25	0.25	Hemolysis.
0.1		0.2	0.05	0.25	0.25	"
0.1	3. C.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	" "
0.1		0.1	0.1	0.25	0.25	" "
0.1		0.05	0.1	0.25	0.25	Hemolysis.
0.1		0.2	0.05	0.25	0.25	"
0.1	4. F.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Hemolysis.
0.1	5. M.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Hemolysis.
0.1	6. M.O.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Hemolysis.
0.1	7. L.	0.3	0.1	0.25	0.25	"

TABLE II.

Complement Deviation.

Immune Sera of Respiratory Strains + Antigens of Meningeal Strains.

Complement. Guinea pig serum 1:40 dilution.	Antigen.		Immune serum (Robinson).	Anti-sheep rabbit serum 1:1,000 dilution.	Sheep corpuscles 1:20 dilution.	Result.
cc.		*cc.*	*cc.*	*cc.*	*cc.*	
0.1	1. D.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	—	—	Hemolysis.
0.1	2. B.H.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	" "
0.1		0.1	0.1	0.25	0.25	Hemolysis.
0.1		0.2	0.05	0.25	0.25	"
0.1	3. N.Y.H.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Some hemolysis.
0.1		0.1	0.1	0.25	0.25	Hemolysis.
0.1		0.2	0.05	0.25	0.25	"
0.1	4. Ch.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Some hemolysis.
0.1		0.1	0.1	0.25	0.25	Hemolysis.
0.1		0.2	0.05	0.25	0.25	"
0.1	5. P.	0.3	0.1	0.25	0.25	No hemolysis.
0.1		0.2	0.1	0.25	0.25	Hemolysis.
0.1	6. F.A.	0.3	0.2	0.25	0.25	"
0.1		0.3	0.1	0.25	0.25	"
0.1	7. L.F.	0.3	0.2	0.25	0.25	"
0.1		0.3	0.1	0.25	0.25	"

Thus it follows that all the sera made by immunizing rabbits with meningeal or with respiratory strains of influenza bacilli contained immune bodies capable of binding complement in the presence of antigens made from both virulent and non-virulent strains. But the sera obtained with the virulent organisms gave binding in higher dilutions than did the sera made from non-virulent bacilli. In other words, the sera obtained by immunizing rabbits with virulent influenza bacilli contained immune bodies capable of uniting with their homologous antigens in comparatively high dilutions, and with heterologous antigens in lower dilutions; while the sera resulting from the inoculation of rabbits with non-virulent influenza bacilli contained less complement binding body for all antigens.

An antigen made from a particular respiratory strain reacted with

TABLE III.

Complement Deviation.

Immune Sera of Meningeal Strains + Antigens of Meningeal Strains.

Complement. Guinea pig serum 1:40 dilution.	Antigen.		Immune serum.		Anti-sheep rabbit serum 1:1,000 dilution.	Sheep corpuscles 1:20 dilution.	Result.
cc.		*cc.*		*cc.*	*cc.*	*cc.*	
0.1	1. B.H.	0.1	1. B.H.	0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	" "
0.1		0.1		0.01	0.25	0.25	Hemolysis.
0.1	2. Ch.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	Some hemolysis.
0.1		0.1		0.01	0.25	0.25	Hemolysis.
0.1	3. N.Y.H.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	" "
0.1		0.1		0.01	0.25	0.25	Hemolysis.
0.1	4. F.A.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0'1		0.1		0.03	0.25	0.25	Hemolysis.
0.1	5. D.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	Hemolysis.
0.1	6. P.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	Hemolysis.
0.1	1. B.H.	0.1	2. N.Y.H.	0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	Some hemolysis.
0.1		0.1		0.01	0.25	0.25	Hemolysis.
0.1	2. N.Y.H.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	" "
0.1		0.1		0.01	0.25	0.25	Hemolysis.
0.1	3. D.	0.1		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.05	0.25	0.25	" "
0.1		0.1		0.03	0.25	0.25	Hemolysis.

a heterologous serum (*i. e.*, from a meningeal strain) in lower dilution than with its own serum. However, still another respiratory strain failed to bind at all with any immune serum. Since these results were obtained in repeated tests with antigens made from the two strains and at different times, they are probably not to be regarded as accidental, but as indicating that the respiratory strains differ among themselves in strength of antigenic power. With this conclusion the protection experiments also agree.

TABLE IV.

Complement Deviation.

Immune Sera of Meningeal Strains + Antigens of Respiratory Strains.

Complement. Guinea pig serum 1:40 dilution.	Antigen.		Immune serum.		Anti-sheep rabbit serum 1:1,000 dilution.	Sheep corpuscles 1:20 dilution.	Result.
cc.		*cc.*		*cc.*	*cc.*	*cc.*	
0.1	1. R.	0.2	1. N.Y.H.	0.1	0.25	0.25	No hemolysis.
0.1		0.2		0.05	0.25	0.25	" "
0.1		0.2		0.03	0.25	0.25	" "
0.1		0.2		0.01	0.25	0.25	Hemolysis.
0.1	2. S.	0.2		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.1	0.25	0.25	Hemolysis.
0.1		0.2		0.05	0.25	0.25	"
0.1		0.3		0.05	0.25	0.25	"
0.1	3. C.	0.3		0.1	0.25	0.25	No hemolysis.
0.1		0.2		0.1	0.25	0.25	" "
0.1		0.1		0.1	0.25	0.25	Hemolysis.
0.1		0.05		0.1	0.25	0.25	"
0.1	4. F.	0.3		0.1	0.25	0.25	Some hemolysis.
0.1		0.2		0.1	0.25	0.25	Hemolysis.
0.1	1. M.	0.3	2. B.H.	0.1	0.25	0.25	No hemolysis.
0.1		0.2		0.1	0.25	0.25	Hemolysis.
0.1	2. Ma.	0.3		0.1	0.25	0.25	No hemolysis.
0.1		0.2		0.1	0.25	0.25	Hemolysis.
0.1	3. S.	0.2		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.1	0.25	0.25	Some hemolysis.
0.1		0.2		0.05	0.25	0.25	Hemolysis.
0.1		0.3		0.05	0.25	0.25	"
0.1	4. C.	0.2		0.1	0.25	0.25	No hemolysis.
0.1		0.1		0.1	0.25	0.25	" "
0.1		0.05		0.1	0.25	0.25	Hemolysis.
0.1	5. L.	0.3		0.1	0.25	0.25	"

While the test of complement deviation brings out a difference between the strains of influenza bacilli isolated from the respiratory tract and those obtained from the meninges, the difference is simply one of degree and not of kind. The sera and antigens made from respiratory strains were both much weaker than were those made from the meningeal strains. This fact is probably explained by the imperfect autolysis of the cultures derived from the respiratory tract and by their inability to produce more than a small amount of protective immune bodies in inoculated rabbits. The antigenic properties of the respiratory cultures are far weaker than are those of the strains isolated from the meninges.

Protection.—In order to determine whether the non-virulent respiratory strains which did not kill rabbits elicit the production of immune bodies, the surviving rabbits were reinoculated at various intervals with virulent meningeal strains.

Ten strains of *B. influenzæ*, isolated from the respiratory tract, were found to be totally unable to afford any protection to young rabbits against a subsequent inoculation with virulent strains. On the other hand, two respiratory cultures which did not kill rabbits in the ordinary lethal dose (one blood agar slant) of the standard virulent strains did protect the animals from a lethal dose of a virulent culture injected after two to four weeks. As was to be expected, sublethal doses of virulent cultures, whether of meningeal or of respiratory origin, protected rabbits against full doses given twelve days to three weeks later.

It is apparent that the respiratory strains are not identical, but that they differ among themselves in the amount of protective immune bodies they are able to develop in rabbits, just as they differ in the amount of complement binding body they produce.

The following protocols illustrate these results.

Protocols.

Experiment I.—Jan. 15. Rabbit, weight 962 gm. Inoculated intravenously with 1 blood agar slant of respiratory strain R of 24 hours' growth suspended in 1 cc. of salt solution.

Jan. 16. Rabbit alive, apparently well.

Jan. 18. Rabbit quite well.

Jan. 30. Rabbit reinoculated intravenously with 1 blood agar slant of meningeal strain N of 24 hours' growth, suspended in 1 cc. of salt solution.

Jan. 31. A. m. Rabbit ill.

P. m., 29 hours after inoculation, rabbit died.

Experiment II a.—Jan. 16. Rabbit, weight 975 gm. Inoculated intravenously with 1 blood agar slant of meningeal strain H of 24 hours' growth, in 1 cc. of salt solution.

Jan. 17. Rabbit dead. Profuse growth of *B. influenzæ* from heart's blood.

Experiment II b.—Jan. 19. Rabbit, weight 975 gm. Inoculated intravenously with ½ blood agar slant of meningeal strain H of 24 hours' growth, suspended in 1 cc. of salt solution.

Jan. 20. Rabbit quite ill.

Jan. 22. Rabbit well.

Feb. 3. Inoculated with one culture of meningeal strain N.

Feb. 5. Rabbit well.

Experiment III.—Jan. 19. Rabbit, weight 970 gm.. Inoculated intravenously with 1 blood agar slant of respiratory strain C of 24 hours' growth, suspended in 1 cc. of salt solution.

Jan. 20. Rabbit well.

Feb. 10. Inoculated intravenously with 1 culture of meningeal strain N in 1 cc. of salt solution.

Feb. 15. Rabbit well.

SUMMARY.

Influenza bacilli isolated from various pathological processes in man differ widely in pathogenic power for animals, especially rabbits. While the cultures derived from the leptomeninges and blood, and rarely from the pneumonic lung are pathogenic, those generally derived from the respiratory tract exhibit little or no virulence for rabbits.

The two types of cultures as indicated by virulence for animals do not differ in kind, but only in degree, in relation to the serological tests of agglutination, complement deviation, and opsonification.

The two types of cultures do, however, differ with respect to their ability to undergo autolysis. While the virulent cultures autolyze almost completely, yielding a turbid supernatant fluid and little sediment, the non-virulent cultures give rise to an abundant sediment and a clear supernatant fluid.

The non-virulent cultures incite far less antibody production in rabbits. Hence, rabbits inoculated with non-virulent strains yield sera possessing low antibody content. Conversely, rabbits inoculated with virulent strains yield sera possessing a higher content of antibody.

In keeping with and possibly because of the low antibody content of the sera of rabbits inoculated with the non-pathogenic strains, the rabbits so treated are not, as a rule, protected against subsequent inoculation with virulent strains.

Influenza bacilli therefore vary in pathogenic effect both for man and animals, but they are not distinguishable by means of serological reactions into different types. Apparently all influenza bacilli belong to one class or race irrespective of origin or virulence.

EXPLANATION OF PLATE 48.

FIG. 1. Large forms of *B. influenzæ* in a film from cerebrospinal fluid in a case of seropurulent leptomeningitis.

FIG. 2. Small forms of *B. influenzæ* in a film from cerebrospinal fluid in a case of seropurulent leptomeningitis. Some phagocytosis.

FIG. 3. Impression from a colony of *B. influenzæ* on a moist blood agar plate. Note long and swollen forms, also polar staining. Meningeal strain.

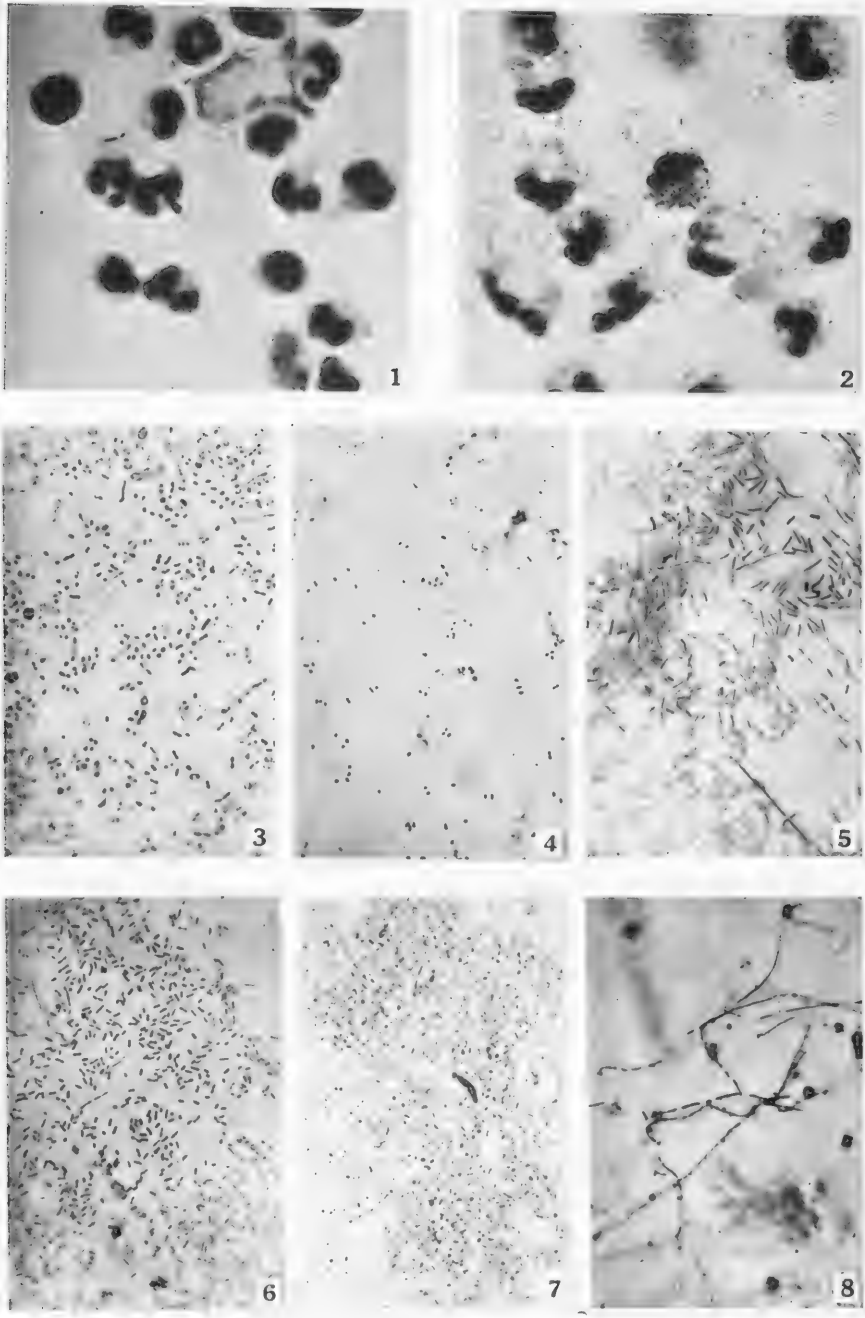
FIG. 4. Characteristic small type culture of *B. influenzæ;* 20 hours' growth. Respiratory strain.

FIG. 5. Large type culture of *B. influenzæ* after 2 years of artificial cultivation.

FIG. 6. Same strain as Fig. 5 from peritoneal cavity of guinea pig 24 hours after inoculation.

FIG. 7. Small type of *B. influenzæ* after 2 years of artificial cultivation.

FIG. 8. Culture of *B. influenzæ* on blood smeared agar, showing interlacing threads.



(Wollstein: Immunological Study of *Bacillus influenzæ*.)

THE MECHANISM OF THE CURATIVE ACTION OF ANTIPNEUMOCOCCUS SERUM.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 49 to 52.

(Received for publication, June 1, 1915.)

In a previous paper[1] I reported on the course of the septicemia in the rabbit which follows an intravenous injection of pneumococci. In brief it is as follows: By making blood cultures at frequent intervals it was found that the course was determined largely by the number and virulence of the bacteria injected. The injection of a relatively small number of pneumococci of low virulence is followed in a few hours by a sterile condition of the blood, and the bacteria do not reappear in the circulation. When large numbers of similar pneumococci are injected or when a small number of a virulent strain is given, the initial decrease in the number of bacteria in the blood takes place less rapidly and completely and, after an interval of five or six hours, an increase occurs. When, however, large numbers of virulent pneumococci are injected, a slight initial fall occurs and death results in a few hours from severe bacteremia.

These results, it was noted at the time, could be completely changed if a small quantity of active antipneumococcus serum was injected. Under these conditions the bacteria even when highly virulent were abruptly swept from the blood. No explanation of the phenomenon was attempted at the time. It is well known that the immune serum is devoid of bactericidal properties *in vitro*. On the other hand, bacteriotropic substances are demonstrable both *in vitro* and *in vivo* and Neufeld and others believe that a relation exists between bacteriotropic value and protective action.[2] The

[1] Bull, C. G., *Jour. Exper. Med.*, 1914, xx, 237.

[2] For previous work and literature on these points the following references are given: Neufeld, F., and Rimpau, W., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1905, li, 283. Boehncke, K. E., and Mouriz-Riesgo, J., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1915, lxxix, 355. Cole, R., *Arch. Int. Med.*, 1914, xiv, 56.

question therefore arises as to what the abrupt disappearance of the pneumococci from the circulation is due and whether it is the result of the action of one or more of the antibodies contained in the blood and the immune serum, and which of them plays the essential part.

EXPERIMENTAL.

The investigation of the pneumococcus was rendered far easier than it otherwise would have been by the fact that the Hospital of The Rockefeller Institute kindly supplied several types[3] of the pneumococcus with their corresponding immune horse serum. Although the majority of the experiments were made with a virulent pneumococcus of Type I, Types II and IV were also employed to supplement and extend the study. Since the investigation of the blood of the rabbits, employed for the tests, during life proved an essential part of the experiments, a brief description of the procedures will be introduced here. They will not be repeated in connection with the illustrative experiments given.

Blood Cultures.—The number of bacteria in the blood stream was determined by taking a small quantity of blood from the heart with a graduated pipette to which a needle was attached by means of a thick-walled rubber tube. A measured quantity of the blood was immediately diluted with a large quantity of sterile physiological salt solution and portions of the dilutions were added to Petri dishes containing sterile defibrinated rabbit blood. The colonies were counted after twenty-four hours' incubation. The plating was done in this manner throughout the work.

Microscopic Examination of the Blood.—For microscopic examination the blood was obtained as described above and heavy films were made on slides. The films were allowed to dry in the air, fixed in methyl alcohol, and stained with a dilute solution of Manson's stain. In this way every bacterium is rendered visible, independently of the thickness of the smear.

Microscopic Examination of the Tissues.—Tissues were prepared for microscopical study by crushing and sectioning. For the crushed preparations, the fresh tissues were finely crushed in tissue crushers, portions of the pulp spread on slides, fixed in a flame,

[3] Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.*, 1913, lxi, 727.

and stained by Gram's method. Sections were stained by Gram's method, eosin and methylene blue, and hematoxylin and eosin.

Immune Serum in Pneumococcus Septicemia in Rabbits.—It has been stated that the immediate effect of the injection of an active immune serum into the blood of rabbits in which many pneumococci are present is to bring about an abrupt disappearance of the bacteria from the circulation, although they may later return. This effect is illustrated by Experiment I in which a bacteremia is produced by the injection of a culture of virulent pneumococcus.

Experiment 1.—4 rabbits received each in the ear vein an intravenous injection of 0.4 cc. per kilo of an 18 hour bouillon culture of a Type I pneumococcus. 2 of the 4 animals received an intravenous injection 1 minute later of the homologous immune serum in the opposite ear vein, while the remaining 2 were untreated. Blood cultures, by the method described, were made from all the animals. The result is summarized in Table I.

TABLE I.

Rabbit No.	Weight.	Immune serum per kilo.	Colonies of bacteria 1 minute after serum injection.	Colonies of bacteria 15 minutes after serum injection.	Remarks.
	gm.	*cc.*			
I	1,550	0.2	1,320,000	0	Bacteremia after 12 hrs.; death in 36 hrs.
II	1,650	0.5	1,250,000	0	Bacteremia; death in 48 hrs.
III	1,850	0	1,260,000	1,100,000	Bacteria did not leave blood; death within 24 hrs.
IV	1,575	0	1,330,000	1,275,000	Same as Rabbit III.

As Experiment I shows, the immune serum brings about a rapid disappearance of the bacteria from the blood. Other experiments directed to the answer of the question indicated that the time required for the blood to be rendered sterile depends, first, on the number of the bacteria injected, and, second and especially, on the amount of serum given. The phenomenon of abrupt removal seems to be independent of the virulence of the pneumococci, although the end result is determined by the virulence. It can be predicted that had the pneumococci been of lower virulence Rabbits I and II would have recovered and the bacteria would not have reappeared in the blood at all.

Mechanism of the Removal Process.

The immediate effect, therefore, of the immune serum is to cause the pneumococci to be rapidly removed from the blood stream. This action is exercised independently of the end result; namely, whether the bacteria are to be permanently suppressed, or whether they reappear later and cause the death of the animals. The question which presents itself for solution is the manner in which the removal is accomplished.

It is known that an immune serum even in the fresh state is devoid of bactericidal action on pneumococci *in vitro*. It was desirable to ascertain the effect of the whole blood under the same conditions. Tests were made by adding to fresh rabbit's blood, either hirudinized or defibrinated, varying quantities of immune serum and a convenient number of pneumococci and then plating the mixture at stated periods during twenty-four hours. It sufficed to add two drops of a bouillon culture, twenty-four hours old, to each cc. of the blood and immune serum mixture.

The effect of the immune serum was to cause great reduction in the number of colonies developing on the plates made from the second to the twelfth hour. The later plates showed increasing numbers of colonies and those made at the expiration of twenty-four hours corresponded with the control plates. The smallest number of colonies on a plate was ten to twenty; sterility was never achieved.

In following with the microscope the changes taking place in the test-tubes it was found that what appeared to be destruction of the pneumococci was actually an agglutination of the bacteria through which the number of colonies was reduced. Definite clumps were discovered in tubes in which the proportion of immune serum was 1 to 500. This fact is noteworthy since the macroscopic agglutination titer of the serum was 1 to 50.

Agglutination and Phagocytosis in Vivo.

It now became desirable to investigate the same points under the conditions of the living body. Experiments 2 and 3 were performed for that purpose.

Experiment 2.—A rabbit having been inoculated by intravenous injection with a small quantity of a bouillon culture of a virulent pneumococcus was permitted to develop a severe bacteremia which required about 10 hours. At that time 1 cc. of immune serum was injected into the ear vein, after which specimens of blood were taken from the heart at intervals of from 30 seconds to 5 minutes. Film preparations were prepared with each specimen, fixed in methyl alcohol, and stained with Manson's stain.

The specimen taken before the serum was injected showed numerous diplococci distributed evenly throughout the blood. The specimen taken 1 minute after the serum was given revealed large clumps of diplococci (Figs. 1 and 2). The next specimen removed showed progressively fewer clumps until the 5 minute specimen was reached, when no diplococci whatever were found.

At the expiration of 30 minutes after the injection of the immune serum the rabbit was killed. The lungs, liver, spleen, and kidney were put through the crusher, and film preparations were made from each. Clumps of diplococci were found in the lungs, liver, and spleen (Figs. 3, 4, and 5), while very few clumps were present in the kidney. The masses of diplococci were sometimes free, but usually they were contained within phagocytic cells and chiefly within polymorphonuclear leucocytes, especially in the lung, liver, and spleen (Figs. 6, 7, 8, and 9). Sections of the organs were also prepared, but they were less informing than the crushed tissues. The sections exhibited, however, groups of leucocytes in the capillaries and other small vessels of the lungs, the sinusoids of the liver, and the blood spaces of the spleen, of which a part enclosed clumps of diplococci (Figs. 12, 13, 14, 15).

Experiment 3.—In this animal the equivalent of a developed bacteremia was induced by injecting into an ear vein the virulent pneumococci derived by centrifugalization from 50 cc. of a twenty-four hour old bouillon culture. After 2 minutes a film of the blood taken from the heart was made and then 1 cc. of immune serum injected. Subsequently film preparations of the heart's blood were prepared at periods of from 30 seconds to 5 minutes.

The results were identical with those described in Experiment 2. The blood film taken before the serum was injected showed many diplococci but no clumps; 30 seconds after the serum injection large clumps were present; the crushed organs showed clumped diplococci within phagocytes.

The two experiments were repeated many times and with unvarying appearances. They were also modified in such a manner as to permit the treated rabbits to survive 1, 2, 3, and 4 hours after the injection of the immune serum. A marked difference was observed in the number and condition of the clumps of bacteria according to the period of survival. The greatest number of free clumps of diplococci were present in the animals killed at the 30 minute period, and the greatest number of phagocytes carrying the clumps were found in the animals killed at one and at two hours (Fig. 10). The preparations of animals killed at two and one-half hours show-

ing the phagocytes containing diplococci are fewer and the form of the bacteria is irregular and the staining faint and poor. Later preparations show very few phagocytes enclosing disintegrated bacteria (Fig. 11). The preparations at four hours contained a small number of free, single diplococci which were the last bacteria to disappear.

So far as the blood phenomena are concerned—the abrupt disappearance of the cocci from the circulating blood, agglutination, and phagocytosis—active and passive immunity are similar. The actively immune rabbits probably possess a tissue immunity which passive immunity does not confer. This point is under investigation.

DISCUSSION.

It is now possible to define clearly the effects exerted by an antipneumococcus serum when it brings about an amelioration of the infective pneumococcus process in rabbits, at least. It may be well to state at the outset that the effects were, in our experiments, limited to the interaction of the specific type or group of pneumococcus and homologous serum. No crossed or heterologous action was ever observed. Although a few typical or illustrative experiments in which Type I pneumococcus and serum were employed are given, similar experiments were made and identical results obtained with pneumococci and serum of Types II and IV.

Setting out with the condition of pneumococcus bacteremia of rabbits, developing on the one hand after a small inoculation of virulent pneumococci and directly produced on the other by an injection of massive quantities of cultures, the first effect of an injection of immune serum is to cause an almost instantaneous agglutination of the diplococci in the blood and the immediate removal of the clump formed by the spleen, liver, lungs, and, to a smaller extent, the kidneys. Probably still other organs participate in this process.

This agglutination and removal by the organs of the pneumococci is quickly followed by a process of active phagocytosis, chiefly through the medium of the polymorphonuclear leucocytes, in the course of which the bacteria are taken up by the cells in enormous

numbers. A single leucocyte may contain from fifty to one hundred diplococci. The act of phagocytosis is directed, apparently, exclusively or almost so against the clumps, while the few single diplococci remain free within the vessels. The act of phagocytosis follows quickly upon the agglutination and removal and, it would appear, never takes place in the blood stream itself; for in the study of hundreds of blood films made from the heart only one leucocyte containing diplococci was encountered. The process extends, however, over several hours and consists of two distinct phases: one the mere englobing of the masses of diplococci, and the other their digestion or disintegration. For the unagglutinated diplococci which remain free retain form and staining powers very much longer than those contained within phagocytes. The diplococci within the phagocytes show at first normal morphology and staining (Figs. 6, 7, 8, and 9), while, later, bizarre forms and imperfect staining are met with (Fig. 11). After a few hours only very few diplococci and those disintegrating are still visible in the phagocytes. Cells other than polymorphonuclear leucocytes play a minor part in the phagocytic process.

The intimate interaction of agglutination and protection which the experiments indicate is emphasized in other ways. I have found that the smallest quantity of immune serum which influences a pneumococcic infection in the rabbit by diminishing the number of diplococci in the circulating blood or by prolonging the life of the animal brings about clump formation *in vivo;* and Avery has noted that in the fractionated and purified antipneumococcic serum the protective bodies are always accompanied by agglutinating substances.

But in order that the agglutinated diplococci may be rendered harmless it is not enough that they shall be removed from the blood by the organs; they must be further withdrawn, apparently, into the phagocytes where they are quickly disintegrated. As the experiments *in vitro* with the whole blood and immune serum showed, the clumped diplococci are capable of further multiplication. Hence, along with the removal of the clumps of diplococci by the organs it is necessary that an accumulation of polymorphonuclear leucocytes in the same organs be brought about simultaneously.

The fact is well known that the intravenous injection of foreign protein causes a leucopenia. I found that the intravenous injection in rabbits of one cc. of antipneumococcus horse serum led, within five minutes, to a reduction of the number of circulating leucocytes by one-half. The low number persisted from three to four hours, after which it rose usually somewhat above the normal. This reduction and rise in number was not participated in by all the white cells but chiefly by the polymorphonuclear leucocytes. Normal horse serum produced similar effects. The injection of bouillon cultures of pneumococci causes a greater fall still in the number of circulating leucocytes and, according to the final result in death or recovery, there was continued depression or later rise.

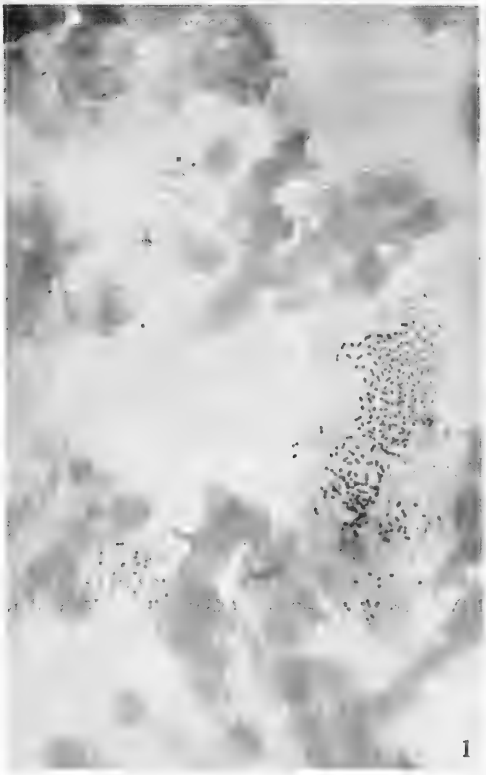
The manner in which the leucocytes react to the foreign protein as contained in the horse serum provides a mechanism for bringing large numbers of polymorphonuclear leucocytes into close proximity with the agglutinated diplococci within the organs. That the leucocytes are not destroyed but merely accumulate in the organ in certain forms of leucopenia has been shown by Goldschieder and Jacob. On the other hand, an homologous immune serum,—for example antipneumococcus serum prepared in the rabbit,—would also bring about agglutination of the diplococci but whether it would equally produce leucopenia has still to be determined. Some other means of attracting leucocytes from the blood and the bone marrow to the capillaries of the organs may be necessary and this means may well have to do with the presence of the clumps of diplococci themselves. But this constitutes a point of further study.

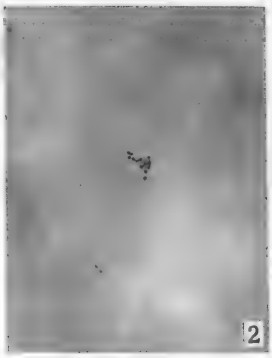
SUMMARY.

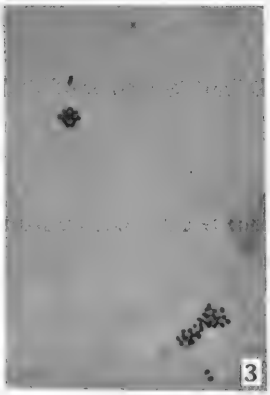
An active antipneumococcus serum causes agglutination of pneumococci *in vitro* and *in vivo*.

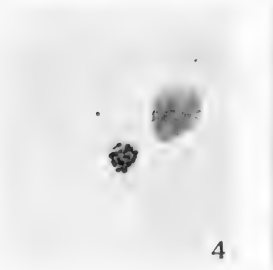
The antiserum acts in far greater dilution in causing agglutination as determined by the microscopic than by the macroscopic test.

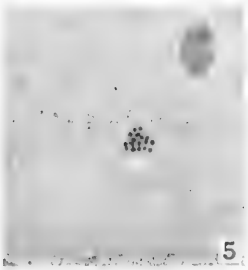
The antiserum when injected into the circulation of rabbits suffering from pneumococcus bacteremia causes a rapid disappearance of the diplococci from the blood. This disappearance is brought about by instantaneous clumping of the diplococci *in vivo* and the removal of the clumps by the liver, spleen, lungs, and possibly other organs.

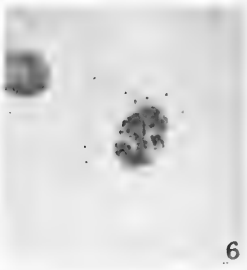




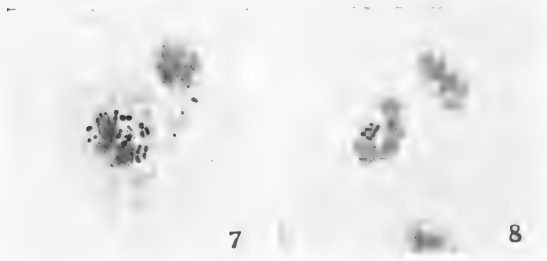


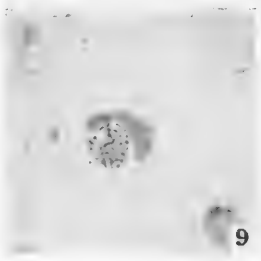


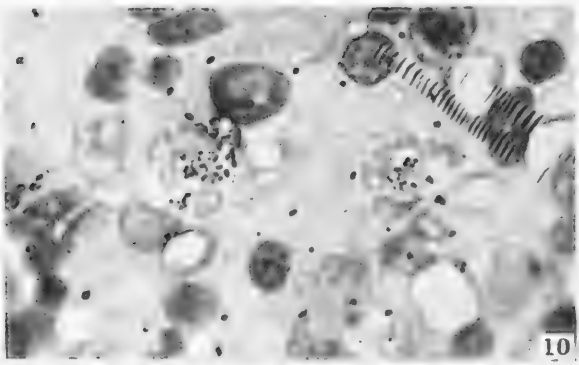


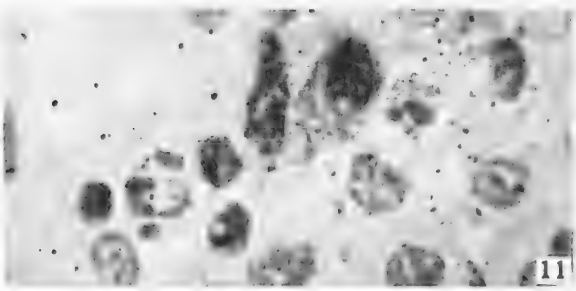


(Bull: Curative Action of Antipneumococcus Serum.)









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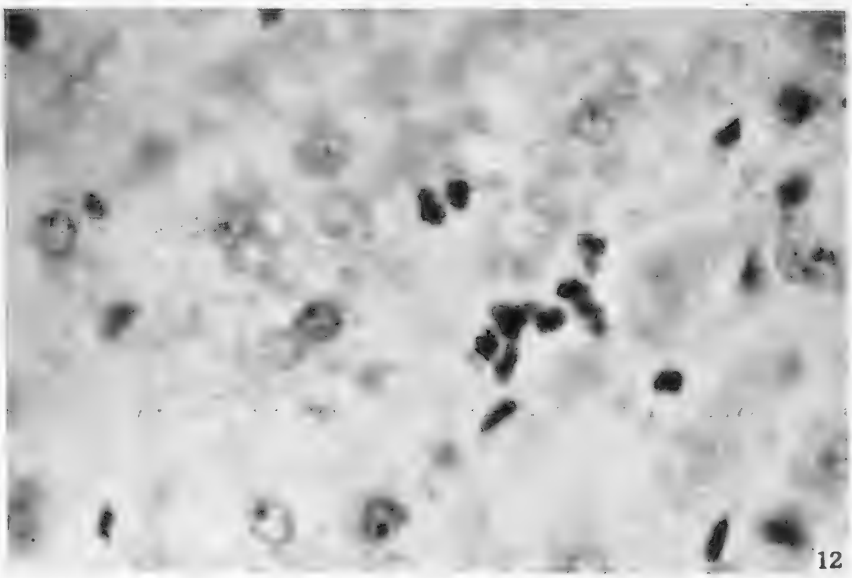

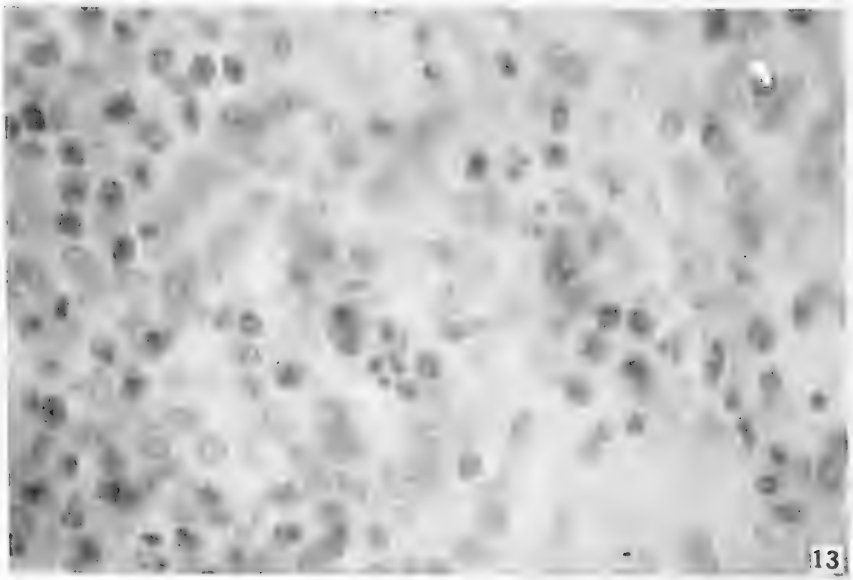

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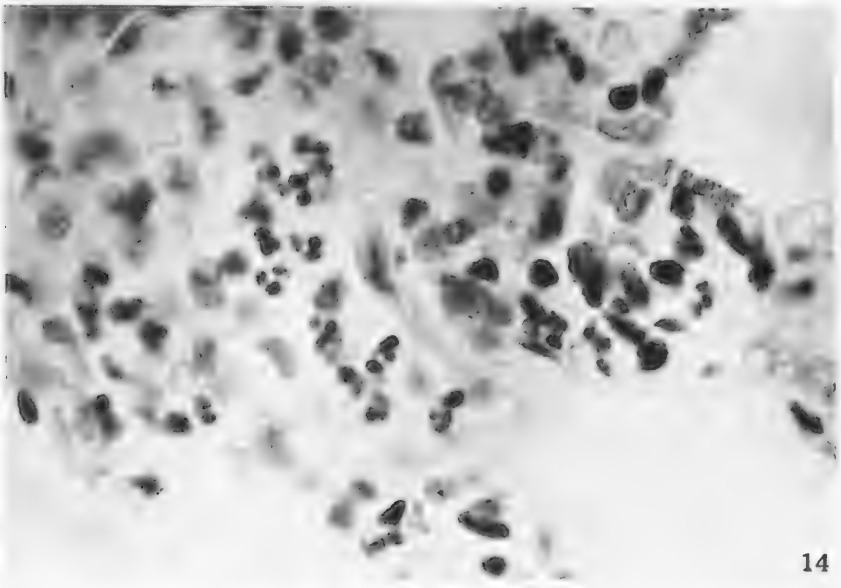

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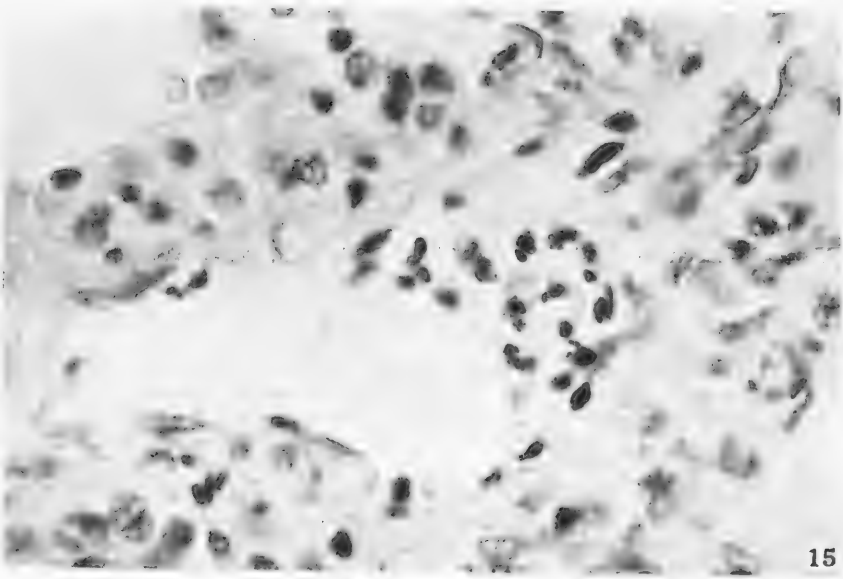

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(Bull: Curative Action of Antipneumococcus Serum.)

The same means which cause the clumping and removal from the blood of the diplococci and their accumulation in the organs cause also leucopenia with accumulation of polymorphonuclear leucocytes in the same organs. The leucocytes act as phagocytes and ingest the clumped diplococci which undergo rapid dissolution within the cells.

The diplococci which fail of agglutination tend not to be phagocyted and persist longer in viable form than the ingested clumps.

The protection afforded by the immune serum is specific for the type organism and is determined by the presence of agglutinins which prepare the pneumococci for removal from the blood and phagocytosis on a large scale.

Normal rabbit blood is devoid of agglutinating and of protective effect against virulent pneumococci.

Agglutination is not merely an incidental phenomenon, but constitutes an essential process in association with phagocytosis in the protection of the rabbit against pneumococcus infection.

EXPLANATION OF PLATES.

PLATES 49 AND 50.

FIG. 1. Clump of pneumococci in heart's blood of a rabbit having a pneumococcic septicemia; specimen was taken 1 minute after 2 cc. of immune serum were given. Gram's stain.

FIG. 2. Clump of pneumococci in heart's blood of a rabbit 1 minute after 0.1 cc. of serum was given.

FIGS. 3, 4, and 5. Clumps of pneumococci in the liver, lungs, and spleen, respectively, of a rabbit 30 minutes after serum had been administered. Gram's stain.

FIGS. 6, 7, 8, and 9. Polymorphonuclear leucocytes containing clumps of pneumococci from the liver, spleen, lungs, and kidney, respectively, of a rabbit having a pneumococcic septicemia. The tissues were removed 1 hour after an injection of immune serum. Gram's stain.

FIG. 10. Crushed preparation from the spleen of a rabbit having a pneumococcic septicemia. 2 leucocytes are present which contain clumps of pneumococci. The tissue was removed 1½ hours after an injection of immune serum had been given. Gram's stain.

FIG. 11. Same as Fig. 10. Tissue removed 2 hours after the serum was given. 1 leucocyte contains a clump of pneumococci. The bacteria stain poorly.

PLATES 51 AND 52.

FIGS. 12, 13, 14, and 15. Sections of tissue from liver, spleen, and lungs, respectively, of a rabbit 2 hours after an intravenous injection of 1 cc. of a bouillon culture of pneumococci. All sections show accumulated polymorphonuclear leucocytes. Stained with hematoxylin and eosin.

A METHOD OF SERUM TREATMENT OF PNEUMOCOCCIC SEPTICEMIA IN RABBITS.

By CARROLL G. BULL, M.D.

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PLATE 53.

(Received for publication, June 1, 1915.)

In a separate paper[1] I have reported experiments which indicate that the removal of pneumococci from the circulation in rabbits and their destruction are determined by three main factors: (1) the agglutination of the bacteria in the blood stream; (2) the assembling of the clumps in the internal organs; and (3) the inclusion of the masses by and digestion within polymorphonuclear phagocytes. In order that these several processes may occur it has been found necessary, in the case of virulent pneumococci, to employ a suitable immune serum.

In this paper I propose to describe still other experiments which bear upon the above conception of the manner of interaction of immune serum and the animal body. It has hitherto been ascertained that the activity of an immune antipneumococcic serum is quite strictly limited. Beyond a given infecting dose of the pneumococci any practicable quantity of immune serum ceases to be protective. Indeed, as the dose of serum is increased, especially when it comes from a foreign species of animal, a point is reached at which the serum becomes deleterious rather than helpful. The precise conditions upon which this change of action depends are not known; it is surmised that a heterologous serum, in small experimental animals especially, may exert a toxic action which becomes noticeable in effect when the dose is relatively large. In any case a striking disparity exists between the unlimited neutralizing action of the antitoxic sera, diphtheria and tetanus sera, for instance, and the strictly limited anti-infectious action of the antibacterial sera, among which the antipneumococcus serum is to be classed.

[1] Bull, C. G., *Jour. Exper. Med.*, 1915, xxii, 457.

Although the literature on the anti-infectious power of antipneumococcic serum is voluminous, the chief recent important contributions to the subject are those of Neufeld and Haendel[2] and of Cole.[3] The former investigators established the fact of the existence of specific types of pneumococci which are subject to influence only by the corresponding immune sera; and the latter, together with his coworkers, besides extending the knowledge of the types of pneumococci and their specific antisera, observed also that up to a certain degree of infection the protective dose of serum is parallel to the quantity of culture inoculated, after which the quantity of serum required to save life is proportionally large, and finally, a degree of infection may be reached against which no amount of serum will protect. For example, 0.2 cc. of serum of Type I will regularly protect a mouse against 0.1 cc. of a Type I culture, no matter how high its virulence. That is the largest dose of culture, however, against which the immune serum will protect, no matter how much serum is employed. 1 or even 2 cc. are no more effective than 0.2 cc. (Cole).

In view of these data, it has been long believed that the animal body supplied a necessary substance which cooperated with the immune serum in overcoming bacterial infections. The nature of this substance has been conjectured merely; there exists no actual knowledge of its nature. The experiments reported in the previous paper referred to[4] seem to indicate quite definitely that the part which the rabbit's body itself plays in overcoming pneumococcus and some other bacterial infections is supplied by the phagocytes. The experiments to be related here bear on this conception of the protective mechanism.

EXPERIMENTAL.

As has just been stated, experiments seemed to indicate that the phagocytic cells function as the chief defensive agents against infection. If this is true, then the limit of the effective action of an immune serum may possibly be extended by giving the phagocytes assistance and providing them sufficient time in which to do their work. We observed that when a rabbit suffering from septicemia is given a

[2] Neufeld, F., and Haendel, *Ztschr. f. Immunitätsforsch., Orig.*, 1900, iii, 159.
[3] Cole, R. I., *N. Y. Med. Jour.*, 1915, ci, 1, 59.
[4] Bull, *loc. cit.*

large injection of immune serum intravenously, the bacteria are agglutinated into massive clumps (Figs. 1 and 2) most of which accumulate in the lungs where they come imperfectly under the influence of the phagocytes and may even, through extensive capillary obstruction, interfere with the circulation. The experiments to follow were performed on rabbits with a strain of pneumococcus of Type I maintained at high virulence by continuous passage through these animals. The corresponding immune serum was prepared in the horse and kindly supplied by the Hospital of The Rockefeller Institute.

The virulence of the culture was not accurately titrated. It was such, however, that one drop of the blood of a rabbit succumbing to infection sufficed to kill in twelve hours a rabbit weighing two kilos. The pneumococci were grown in beef infusion broth and were eighteen to twenty-four hours old when used. When large quantities were to be injected the bacteria were thrown out of the culture in the centrifuge, the greater part of the fluid was poured off, and the remainder suspended for injection. The bacteria were not washed. The injections both of bacteria and serum were made intravenously.

Experiment 1. Effect of Large Serum Injection in Rabbits Suffering from Severe Pneumococcus Septicemia.

Rabbit A.—Weight 1,800 gm. 0.1 cc. of culture given. 8 hours later the blood contained large numbers of pneumococci. 4 cc. of immune serum injected. The animal died within 1 minute of respiratory failure.

Autopsy.—Large clumps of pneumococci in the heart's blood; the lungs were distended, and many clumps of bacteria were in the vessels; clumps of bacteria also in the vessels of the choroid plexus (Figs. 1 and 2).

Rabbit B.—Weight 1,600 gm. 0.1 cc. of heart's blood of a rabbit just dead of pneumococcus septicemia given. 6 hours later the blood revealed the existence of a severe septicemia with the diplococci uniformly scattered throughout. 2 cc. of immune serum were injected and films from the heart's blood were made every minute for 3 minutes.

The immediate effect of the serum was to produce collapse of the animal attended by labored respiration and urination. The 1 minute film showed many large clumps of bacteria, the 2 minute fewer clumps, and the 3 minute few small clumps. The rabbit survived, temporarily recovered, but died 6 hours later. The blood and organs contained many pneumococci.

Rabbit C.—Weight 2,200 gm. The sediment from 50 cc. of a bouillon culture injected. 2 minutes later 10 cc. of immune serum given in opposite ear vein. The blood was quickly cleared of the pneumococci. Death occurred after 12 hours.

Autopsy.—Lungs edematous, emphysematous, and hemorrhagic. Small hemorrhages in surface of the kidneys and peritoneal surface of the intestines. The blood, lungs, spleen, and liver contained very great numbers of pneumococci.

Rabbit D.—Weight 2,300 gm. The sediment from 50 cc. of bouillon culture injected. No further treatment. Death in 4½ hours. The blood and organs contained very many pneumococci.

The experiments indicate that when the blood of the rabbit contains a very large number of pneumococci the intravenous injection of a large amount of immune serum causes certain definite effects. In the first place, the effect may be to cause almost immediate death. This accident results from the rapid agglutination in large clumps of the pneumococci in the circulation and the massing of the clumps in the lungs and brain where, acting as emboli, they produce respiratory failure and death. In the second place, the blood may be temporarily cleared of the bacteria and life be prolonged. Finally, however, the life of the animal is not spared, as the bacteria reinvade the blood and cause death.

From the foregoing it appears that the formation of large clumps is a disadvantage to the animal, since they tend to be held back chiefly in the lungs, the circulation of which they obstruct; and, besides, the large bacterial masses are not readily phagocyted.

These disadvantages can be avoided by the injection of small quantities of immune serum which produce clumps containing twenty to thirty pneumococci and to their quite regular distribution in the lungs, liver, and spleen, where they come under the influence of phagocytes under favorable conditions. Once removed from the circulation and lodged in the organs, the effect of a larger quantity of serum was studied.

Experiment 2. Effect of First Small and Later Larger Doses of Immune Serum in Pneumococcus Septicemia.

Rabbit A.—Weight 1,800 gm. The sediment from 75 cc. of a bouillon culture given. Immediately 0.5 cc. of immune serum injected into opposite ear vein. At the examination 25 minutes later there were no bacteria in the blood. 5 cc. of immune serum injected. Animal showed no symptoms for 2 hours, after which symptoms appeared, and death occurred at end of 5 hours.

Autopsy.—The lungs were edematous and hemorrhagic. Hemorrhages in surface of kidneys and peritoneal coat of intestine. The blood was free of diplococci and very few were found in the spleen, lungs, and liver. Almost all had been destroyed.

Rabbit B.—Sediment from 75 cc. bouillon culture administered. No treatment. Died in 6 hours. Blood and organs teeming with bacteria.

The experiment recorded under Rabbit A was repeated several times but without changing the end result. In spite of the practical destruction of the pneumococci, death quickly resulted. The appearance of the organs, and especially the hemorrhages, were taken to indicate a severe grade of intoxication,[5] and this effect was provisionally related to the large second dose of serum leading to too rapid disintegration of the diplococci. Hence it was decided to bring about a more gradual disintegration of the bacteria if possible.

Experiment 3. Effect of Repeated Small Doses of Immune Serum in Pneumococcic Septicemia.

Rabbit C.—Weight 2,000 gm. The sediment from 150 cc. of a bouillon culture given. Two minutes later 0.5 cc. of immune serum injected. At the end of 30 minutes the bacteria had left the blood and 1 cc. of the serum was administered. From now on 1 cc. of the serum was administered every 2d hour. Although symptoms appeared at the end of the first 2 hours they disappeared and the rabbit was in good condition until the 22d hour, when restlessness and rapid respiration followed by rigidity of hind leg developed. Later opisthotonos supervened. A lumbar puncture yielded cerebrospinal fluid containing many diplococci. Death occurred in the 28th hour.

Autopsy.—Blood, spleen, liver, and lungs were free of bacteria. The meninges were inflamed and contained very large numbers of pneumococci. Death obviously resulted from meningitis.

Rabbit D.—The sediment from 100 cc. of bouillon culture was given. No treatment. Died in 4 hours. The blood and organs contained very large numbers of diplococci.

The experiment described in Rabbit C was made several times but always with the same final result. The success of the experiment was frustrated by the intervention of the meningitis. The possibility is not excluded that this complication may itself be prevented by bringing a proper dose of the immune serum into the meninges which are not reached from the circulating blood.[6] The effect of reducing the infecting dose of bacteria was next tried. The sediments from 75 cc. and from 50 cc. of bouillon culture only delayed the onset of meningitis. The sediments from 35 cc. of culture gave partially successful results.

[5] Sprunt, T. P., and Luetscher, J. A., *Jour. Exper. Med.*, 1912, xvi, 443.
[6] Flexner, S., *Jour. Am. Med. Assn.*, 1913, lxi, 447.

Experiment 4.

4 rabbits weighing from 1,900 to 2,200 gm. were given the sediment from 35 cc. of bouillon cultures. 2 minutes later 0.5 cc. of immune serum were injected into the opposite ear vein. 30 minutes later and every 2d hour thereafter 1 cc. of the serum was given. This was continued up to the 56th hour. The final results are shown in Table I.

TABLE I.

Rabbit 1.		Rabbit 2.		Rabbit 3.		Rabbit 4.	
Time.	Observation.	Time.	Observation.	Time.	Observation.	Time.	Observation.
hrs.		*hrs.*		*wks.*		*hrs.*	
67	Right hind leg paralyzed	6	Diarrhea		In a light stupor for 1st day. No other symptoms	50	Both forelegs paralyzed.
		24	"				
83	Both hind legs completely paralyzed; severe opisthotonos; very restless and excitable	48	Diarrhea improved. Rabbit showed no further symptoms			53	Severe opisthotonos, restless, excitable, breathing labored.
				3	Agglutinating titer of serum, 1:125		
96	Comatose; lying on side; breathing labored	3 wks.	Serum agglutinates pneumococci in a dilution of 1:150	6	Living and in perfect condition	60	Comatose; lying on side.
122	Died					80	Died.
	Autopsy.—Brain and cord intensely injected. Smears from surface of brain and lateral ventricles showed very many pneumococci. No pleurisy, no pericarditis. Blood, lungs, liver, and spleen free of bacteria	6 wks.	Living and in perfect condition				*Autopsy.*—Same as Rabbit 1.

Experiment 4 shows that it is possible by employing properly graduated doses of an immune serum to cure rabbits of a massive pneumococcus infection. The experiment indicates also that it is only the chance diplococci excluded from the influence of the circulating immune serum which escape destruction and multiply. It is of interest to know that the probability of the pneumococci escaping the destructive action of the serum is reduced by one-half by diminishing the infecting dose to a point which is, however, still massive according to the grade of virulence of the strain of pneumococcus employed.

Experiment 5. A Comparison of the Three Methods of Treatment Employed.

2 rabbits were used for each method. The infecting dose was the sediment from 20 cc. of bouillon cultures. 2 rabbits were treated, as in Experiment 4,

with frequent small doses of serum; both recovered. 2 were treated first with a small dose of serum and then with a large dose. They died. 2 were given one large dose of serum immediately after the infection; both died of septicemia.

DISCUSSION.

The experimental data presented in the foregoing pages should be considered from several points of view: from their bearing on experimental pneumococcus infection in the rabbit, and from their bearing on the theory of the anti-infectious action of immune serum in general.

The most striking fact regarding the experimental pneumococcus infection is the one that a single large dose of the immune serum given at the beginning of the infection is far less effective in overthrowing the infection than small repeated doses of which the maximal amount may not equal the single large quantity unsuccessfully employed.

The reason for this disparity has also been made clear in large part. The serum does not protect by a process of neutralization of a true toxin as antidiphtheritic serum does; or if any neutralization occurs it is a minor, and not the decisive process. The anti-infectious serum performs two things: it brings about an agglutination of the bacteria, and it prepares them for phagocytosis in the organs. Hence, whatever favors this process will be beneficial, and whatever hinders it will be detrimental. A certain concentration of the serum likewise promotes the assembling of the leucocytes in the organs.[7] On the other hand, higher concentration causes the formation of such large clumps as to escape phagocytosis. The free bacteria then quickly multiply, escape into the blood, and cause fatal infection. An excess of serum acts disadvantageously in another way not yet explained. Even when, through a small dose of serum, the small clumps have been formed in the blood and removed by the organs, a large following dose of the serum brings about a fatal issue. In this instance, the bacteria do not begin to multiply and invade the organs. The blood and the organs may be quite or nearly sterile. Death appears to result from intoxication. But just how the serum acts in producing the intoxication has not been determined.

When the pneumococci have all been destroyed in the organs

[7] Bull, *loc. cit.*

through the operation of the small doses of the serum repeated at intervals, only part of the rabbits are saved. The effect of the serum thus administered is clearly to provoke the destruction of the bacteria under conditions which avoid intoxication. But only those pneumococci are destroyed and rendered harmless that come under the direct influence of the serum which can reach all essential parts of the body except one; namely, the subdural space. When, under any circumstances, the pneumococci reach the subdural space they are not restrained by the treatment but develop rapidly and in great numbers and thus cause a fatal meningitis. The conditions leading to the meningitis are two: (a) large dosage and (b) survival for a sufficiently long period of time. The dose must be larger than the small doses of serum capable of destroying in a certain time period, and the animal must survive even the large doses about 20 hours in order that the meninges may become infected.

But the quantity of virulent pneumococci which rabbits can be made to support under the influence of the method of repeated serum injections is still so very large that the question may be raised whether the limits of activity of this anti-infectious serum have not been greatly underestimated. It remains, of course, to be determined whether still other anti-infectious sera are capable of having their powers enhanced by a similar method of administration. In any case, the subject is one that calls for restudy and perhaps revision.

On the other hand, the experiments affirm nothing as to the efficacy of the method in the serum treatment of lobar pneumonia in man. It may be supposed that so far as the pneumococci in the circulating blood in lobar pneumonia are concerned, small doses of the serum would suffice to bring about their removal. What effect the small doses might have upon the pneumococci in the lungs cannot be predicted. But since the method is one that is readily carried out in man it will doubtless receive attention in due time.

SUMMARY.

The treatment of pneumococcic septicemia in the rabbits by large doses of immune serum is detrimental, since the serum causes the

formation of large clumps of bacteria in the blood which are taken out chiefly by the vessels of the lungs in which they accumulate and impede the circulation.

The large doses of serum are also detrimental when they follow upon small ones through which the small clumps formed are deposited in the spleen, liver, and other organs. In this instance, the large amount of serum leads to the destruction of the pneumococci under conditions which promote an intoxication. The precise mechanism of this action is not known.

The treatment of pneumococcic septicemia in rabbits by small repeated doses of immune serum can be successfully carried out. The number of pneumococci capable of being brought to destruction through phagocytosis in the organs in this way is very great.

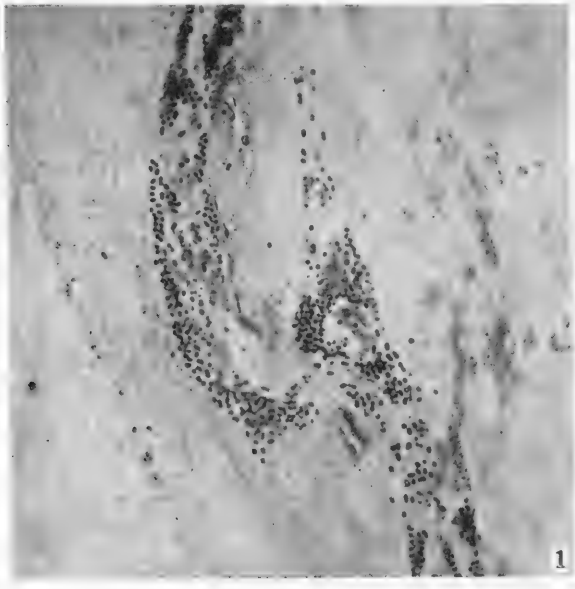
Not all the rabbits treated with small repeated doses of the serum survive. Those that succumb do so not to a general infection but to a pneumococcus meningitis. The explanation of this phenomenon is simple. When the number of pneumococci originally inoculated is very great a small number penetrate into the subdural space. Those in this space do not come under the influence of the serum, hence they are not agglutinated and prepared for phagocytosis, whence they multiply and set up a fatal meningitis.

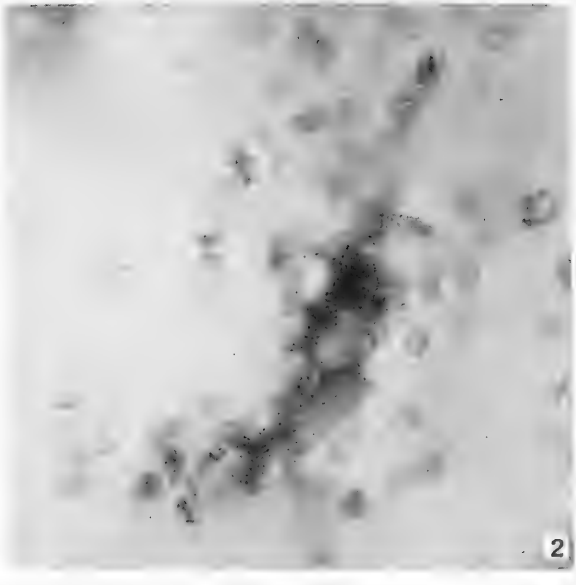
The activity of the immune serum administered in this way against virulent pneumococci is so great that a revision of our notions in the limit of powers of the anti-infectious sera seems necessary. It is patent that the problem is not simply a relation between quantity of immune bodies and number of bacteria. It is more complex than that conception indicates. The factor of the leucocytes and the degree of their possible activities under the conditions of the experiment come into play. Hereafter, in defining the mode and power of action of anti-infectious sera the condition of cooperation of the body-forces will have to be more strictly considered.

EXPLANATION OF PLATE 53.

FIG. 1. A large mass of pneumococci in a blood vessel. Tissue was taken from the region of the choroid plexus of a rabbit dying 1 minute after receiving 3 cc. of immune serum. The rabbit had a severe pneumococcus septicemia when the serum was given.

FIG. 2. A large clump of pneumococci. Preparation from the lung of the rabbit described in Fig. 1.





(Bull: Treatment of Pneumococcic Septicemia in Rabbits.)

THE FATE OF TYPHOID BACILLI WHEN INJECTED INTRAVENOUSLY INTO NORMAL RABBITS.

By CARROLL G. BULL, M.D.

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PLATE 54.

(Received for publication, June 1, 1915.)

The course of typhoid bacillus infection in the rabbit has been followed by many investigators. Certain facts are established: The typhoid bacilli injected into the blood do not long remain in the circulation; the gall bladder frequently becomes invaded quickly after the injection; and the bacilli survive and multiply there for a long period of time.[1] Ultimately they may disappear from all parts of the body except the gall bladder.

The present study was undertaken to determine more accurately than had hitherto been done the manner in which typhoid bacilli are removed from the blood of normal rabbits. This study was part of a more general study of the mechanism of bacterial immunity in the rabbit,—whether native or acquired. The rabbit may be regarded as possessing a high degree of natural immunity for the typhoid bacillus, and the fate of the bacilli injected was traced from the blood through the various organs in arriving at an explanation of their disappearance.

Source of the Cultures.

The strain of typhoid bacilli used in the mass of the work was obtained from a capsule of Besredka's sensitized vaccine kindly supplied by Captain H. J. Nichols of the Medical Corps, U. S. Army. Three other typical strains from widely different sources were used to check the results obtained with the Nichols strain. All four strains had been under artificial cultivation for some time and should

[1] Nichols, H. J., *Jour. Exper. Med.*, 1914, xx, 573.

be considered as essentially non-virulent, although death of the animal due to intoxication sometimes ensued. The bacilli used for inoculation were cultivated on plain agar and used when twenty-four hours old. For injections they were washed from the medium with 0.85 per cent sodium chloride solution.

Disappearance of Bacilli from the Blood.

From one-thirtieth to one-fiftieth of the bacilli from an agar slant were injected into the ear vein and Petri plates were made from the heart's blood at various intervals, beginning as early as thirty seconds after the injections. It was found that the first specimen taken always contained the largest number of bacilli and that they left the blood with remarkable rapidity. Cultures made ten minutes after the injections contained only a few colonies. Specimens taken fifteen to twenty minutes after injecting the bacteria were often sterile. In some cases a few colonies developed in cultures made several hours after the inoculations or at the time of the death of the animals. Even when the bacilli from an entire agar slant were given and death resulted in two hours the blood was frequently sterile. The following instance will serve as a typical experiment.

Experiment 1.—A rabbit weighing 2,000 gm. was given 1/10 of a 24 hour agar slant of typhoid bacilli in the ear vein. Blood cultures made from the heart at stated intervals gave the results indicated in Table I.

TABLE I.

Time after injection. *min.*	No. of colonies per cc.
1	10,000,000
2	2,500,000
5	100,000
15	40
20	1

In several experiments of this kind the same general results were obtained. In some instances the bacilli left the blood somewhat more slowly than in others, but the variation was a matter of a few minutes only.

The abrupt disappearance of typhoid bacilli from the blood stream was investigated. Why should the bacilli leave the blood

so much more rapidly than other bacteria, namely, streptococci, pneumococci, or dysentery bacilli of the Shiga type, which were studied? If the process was merely a matter of filtration by the capillary systems of the various organs as Wyssokowitsch[2] concluded, this difference should not exist. The notion prevailing has been that the typhoid bacilli are destroyed (dissolved) by the rabbit's blood within a short time. Indeed, it is known that normal rabbit's whole blood or serum *in vitro* kill the bacilli in relatively brief periods of time. Our next inquiry was directed to the organs to determine whether the bacilli taken out of the blood accumulated in them.

Relation of Bacilli to the Tissues.

From one-thirty-fifth to one-fiftieth of the growth from an agar slant was injected into the ear vein of normal rabbits and specimens of blood were taken for culture from the heart one minute after the inoculation to determine the number of bacilli injected, and again at ten minutes to make sure that the bacilli had left the circulating blood. The rabbits were then killed by a stroke on the neck at ten, twelve, or twenty minutes, and the various organs removed and crushed finely in tissue crushers, after which definite quantities of the pulps were thoroughly shaken in sterile salt solution, and plated. The results of this test are given in illustrative Experiment 2. It may be stated here, however, that in no instance were as many bacilli found in any of the organs per unit of measure as had been present in the blood, and moreover that most of the tissues contained very few bacilli at all.

Experiment 2.—A rabbit was given 200,000,000 typhoid bacilli, as determined by test plating, into the ear vein, and the following data were determined: blood from the heart 1 minute after the injection gave 3,000,000 colonies per cc.; 6 minutes after, 80; and 16 and 20 minutes after, no colonies per cc. The tissues removed at 21 minutes gave colonies per cc. of crushed tissue pulp as follows: spleen, 2,000,000; liver, 1,600,000; lung, 100,000; mesenteric lymph node, 1,000; skeletal muscle, brain, and other tissues, comparatively very few. The weights of the organs were determined and the total number of bacteria recovered, with the finding that it was far below the actual number injected. In none of the organs was the number of colonies per unit of measure as great as the number originally found in the blood.

[2] Wyssokowitsch, W., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1886, i, 3.

From this and similarly conducted experiments it was concluded that the bacilli did not accumulate in the tissues, and it was therefore considered desirable to follow the course of events in an organ for several hours after the bacilli had been injected into the blood. The liver was selected for this study since it is possible to remove portions of the organ at various intervals over a period of time. A typical result is given in Experiment 3.

Experiment 3.—A rabbit was given 1/35 of an agar slant of a culture of typhoid bacilli into the ear vein and estimates were made as follows: Blood 1 minute after the injection gave 15,000,000, and 10 minutes after, 10 colonies per cc.; liver 3 minutes after the injection gave 12,000,000, 14 minutes 6,000,000, 1 hour 700,000, 2 hours 80,000, and 3 hours 1,000 colonies per cc. of crushed pulp.

The data supplied by Experiment 3 support, on first examination, the prevailing notion that certain bacteria and, in this instance, typhoid bacilli fall a ready victim to the destroying and presumably the dissolving effect of the blood, whether in the general circulation or in the capillaries of the organs. This view is based in large part on the classical studies of Wyssokowitsch, and is upheld by the well known fact that fresh serum and the whole blood of rabbits are highly destructive *in vitro* to the typhoid bacilli. Without, however, adopting the usual explanation of the disappearance of the typhoid bacilli from the blood and organs, as illustrated by the experiments on the liver, it was deemed desirable to repeat certain of the experiments on the bactericidal action of normal rabbits' blood.

Action of the Serum and Whole Blood of the Rabbit in Vitro on Typhoid Bacilli.

Agglutinins.—The serum of normal rabbits was tested for agglutinating value. The bacilli were grown upon agar slants and washed off with 5 cc. of normal saline solution. One drop of the suspension of bacilli was added to one cc. of the serum dilution. The agglutinating value of normal serum varied from zero in full serum to positive in 1 to 100 dilution. About one-fifth of the sera examined were devoid of demonstrable agglutinins. The agglutinins are thermolabile and are destroyed at 56° C.

Bactericidal Power.—The fresh serum obtained from the heart's

blood after coagulation was diluted with saline solution and from 2,000 to 4,000 typhoid bacilli (estimated by test plating) were added to one cc. of the dilution. The mixtures in test-tubes were incubated at 37° C. for two hours and the entire contents of the tubes were plated. In order to eliminate the clumping of the bacilli and adhesion to the sides of the tubes, melted agar was poured into them after the plating and they were incubated and examined.

The results can be stated as follows: The sera of different rabbits vary considerably in destructive effect on typhoid bacilli. Full serum and serum diluted 1 to 10 usually destroyed all the bacilli. Serum diluted 1 to 50 usually caused diminution but never complete destruction of the bacilli. The defibrinated whole blood and hirudinized blood act in a manner similar to the serum.

There can, therefore, be no doubt that fresh rabbit serum and the whole rabbit blood destroy considerable numbers of typhoid bacilli *in vitro*. Whether the same means operate in the destruction which takes place *in vivo* remains to be determined.

Fate of Typhoid Bacilli in the Blood and Organs of Inoculated Rabbits.

The experiments performed with the pneumococcus[3] suggested that a similar study be made of typhoid bacilli injected into the circulation of rabbits, especially in view of the fact that while rabbits readily succumb to typhoid intoxication they are highly resistant to typhoid infection. Experiment 4 will serve as an illustration of this class of tests.

Experiment 4.—An agar slant of typhoid bacilli was suspended in about 5 cc. of salt solution and injected into the ear vein of a normal rabbit. Specimens of the heart's blood were taken and films prepared at 30 seconds, 1, 2, 3, 5, and 7 minutes. The films were stained by Manson's method. Clumps of bacilli were found even in the first film (Fig. 1). The second film showed a larger number of clumps, while the number diminished in the next specimen, and none were found in the last, or 7 minute, specimen. At the end of 7 minutes the rabbit was killed by a stroke on the neck and the organs were immediately removed and finely crushed in tissue crushers. Films were prepared from the pulp and stained by Manson's method. Microscopical examination of the slides showed that clumps of bacilli had accumulated in the capillaries, sinusoids, and blood spaces

[3] Bull, C. G., *Jour. Exper. Med.*, 1915, xxii, 457.

of the various organs, especially the liver, lungs, and spleen, and a large proportion of the clumps had already been phagocyted by the polymorphonuclear leucocytes (Figs. 2, 3, and 4) which had accumulated in the organs following the injection of the bacteria. Free and unclumped bacilli were also found.

This experiment was repeated in its essential aspects several times with wholly concordant results. The experiment was varied in such a manner as to permit the inoculated rabbits to survive for different periods of time, after which the blood and organs were examined. It was found that the largest number of leucocytes enclosing clumps of bacilli were present in the organs of animals killed from thirty to ninety minutes after inoculation. The organs of animals permitted to survive three or four hours still contain many leucocytes, but very few leucocytes containing distinct bacteria are met with. Leucocytes containing granules of disintegrated bacilli or bacilli which have lost power of staining may be found in small number. On the other hand, the three hour specimens still show a certain number of unagglutinated bacilli, outside cells with staining properties unimpaired (Fig. 5).

Still another variation of the experiment was to remove portions of the liver from ten minutes to two hours after injecting the bacilli. The findings just described were corroborated by this procedure. Specimens taken from ten to ninety minutes contained many phagocyting cells; while later specimens showed fewer phagocyting cells and finally cells which enclosed only disintegrating bacteria.

DISCUSSION.

If we review the findings described in this paper we shall arrive at somewhat conflicting results as to the manner in which typhoid bacilli are disposed of, respectively, by the body and the blood of the rabbit.

Directing attention first to the phenomena observed outside the body it may be affirmed, in keeping with usual knowledge, that the fresh blood serum as well as the fresh whole blood of the rabbit is capable of destroying, apparently by a process of solution, considerable numbers of typhoid bacilli. There is no reason, moreover, to doubt that the process of destruction in this instance is the

common one of bacteriolysis in which amboceptor and complement play the decisive part.

But the essential question at issue is not the extracorporeal but the intracorporeal method of destruction of typhoid bacilli. It is upon that point that light is especially needed. To apply directly the results of test-tube experiments to the explanation of what takes place in the body itself has not proven wholly illuminating. We already know that typhoid bacilli may appear and survive in the blood of human typhoid fever patients at a time when the shed blood is highly bacteriolytic for the bacilli.

The observations which this paper records indicate a wide disparity between the processes involved in the destructions of the bacilli in test-tubes and in the living body. In the latter, the bacilli introduced into the blood are quickly agglutinated, after which they are removed by the organs. In the interstices of the organs they come into close relation with polymorphonuclear leucocytes (themselves assembled in the organs as result of the bacterial injection) which englobe and destroy them. There is no evidence at hand which connects ordinary bacteriolysis with *intra vitam* destruction of typhoid bacilli. The unagglutinated and unphagocyted bacilli in the organs resist longest and stain best; and complement has yet to be detected in the circulating plasma.

This view of the process of *intra vitam* destruction of typhoid bacilli may serve to explain the fact that typhoid bacilli sometimes circulate in the blood of typhoid patients. It is known that bacilli cultivated from the circulating blood are often inagglutinable. We have found no indications that phagocytosis of the bacteria studied by us takes place in the blood or on a grand scale in the unagglutinated state. Hence, as the bacilli cannot be agglutinated and removed by the organs and also cannot be phagocyted in the blood stream, they continue to circulate, under some conditions, until they are removed and destroyed by the phagocytes.

The indications therefore are that in the body the destruction of typhoid bacilli by means of bacteriolysis does not take place. The question arises, however, whether in the tests of the survival of the bacilli in the pulp the conditions produced do not render the operation

of the bacteriolytic processes impossible. To test this point, crushed liver, spleen, and kidney were added to fresh normal rabbit sera just previous to introducing the typhoid bacilli. The test-tubes were incubated for two hours, after which plates were made. It developed that the action of the spleen and kidney pulps was negligible, while the liver pulp caused complete inhibition of bactericidal effect. Further tests indicated that it is the biliary constituent of the liver that is responsible for this action, since bile in quantities themselves non-hemolytic inhibits the activity of a hemolytic system apparently through its anticomplementary effect.

SUMMARY.

Typhoid bacilli are agglutinated promptly in the circulating blood of normal rabbits and quickly removed from the blood stream.

The clumped bacilli accumulate in the organs and are taken up by assembled polymorphonuclear leucocytes in the liver, spleen, and possibly other organs.

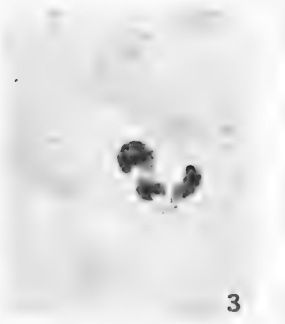
The phagocyted clumps of bacilli are digested and destroyed by the phagocytes.

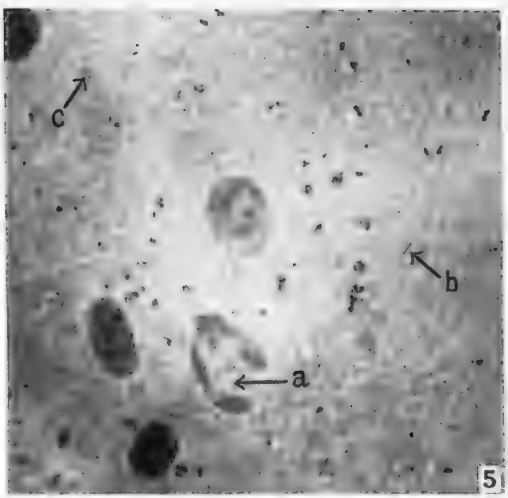
Hence, destruction of typhoid bacilli *intra vitam* is brought about by an entirely different process than is the destruction by serum and whole blood *in vitro*. While the latter is caused by bacteriolysis, the former results from agglutination and intraphagocytic digestion.

Lysis by fresh blood serum is not appreciably affected by spleen or kidney pulp, but it is inhibited by liver pulp. The action of the liver is referable to its biliary constituents, which exert anticomplementary action.

Probably in certain examples of typhoid fever in man the typhoid bacilli in the circulating blood being inagglutinable cannot be removed by the organs and hence are not phagocyted and destroyed.

The observed disparity between the ready destruction of typhoid bacilli by serum and shed blood and the resistance sometimes offered by the bacilli in the infected body is explained by the essential differences in the destructive processes in operation within and without the body.





(Bull: Fate of Typhoid Bacilli Injected into Normal Rabbits.)

EXPLANATION OF PLATE 54.

FIG. 1. Heart's blood from a rabbit containing 2 clumps of typhoid bacilli. The specimen was taken 30 seconds after the bacteria were injected into the ear vein. Stained by Manson's method.

FIGS. 2, 3, and 4. Polymorphonuclear leucocytes from lung, liver, and spleen, respectively, containing clumps of typhoid bacilli. The tissues were removed from the animal 30 minutes after the bacteria were injected. Manson's stain.

FIG. 5. A smear of crushed liver tissue. The specimen was removed from a rabbit 2½ hours after an intravenous injection of typhoid bacilli. (a), leucocyte containing disintegrated bacilli; (b) and (c), free bacilli staining deeply. Manson's stain.

THE AGGLUTINATION OF BACTERIA IN VIVO.

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Among the factors to which bacterial immunity is ascribed the phenomenon of agglutination occupies the most subordinate place. Indeed, the general view held by immunologists is to the effect that however valuable agglutination may be *in vitro* in identifying bacteria or discovering specific forms of infection, yet the process plays no essential part in the protection of animals as such. The appearance of agglutinins in the sera or body fluids of infected individuals or inoculated animals is considered a sign of the existence of a greater or less degree of immunity; the agglutinins themselves being merely incidental accompaniments of the true immunity factors,—lysins, bacteriotropins, and antitoxins. We have, however, made certain observations within the last few months which indicate that agglutinins play quite an important part in at least certain instances of active and passive immunity.

In reporting his classical work on the specificity and practical application of the phenomenon of agglutination, Gruber[1] expressed the opinion that agglutinins were quite essential properties of an immune serum. He believed that the phenomenon of agglutination was due to the fact that the agglutinins (Glabrificine) increased the viscidity of the bacterial bodies and caused them to adhere to one another. Gruber advanced the idea also that this increased viscidity aided in the englobement of the bacteria by the phagocytes, and would probably explain the accumulation of bacteria in the organs when they were injected into the circulation. Gruber's hypothesis has never been further developed or proved by himself or others.

Metchnikoff[2] contends that agglutinins play no rôle, however small, in active or passive immunity processes. "The phenomenon of agglutination is of no great importance from the point of view of natural immunity."[3] "We have already given the arguments which render it impossible for us to attribute to the agglutinative property of the fluids of the body any rôle, however unimportant,

[1] Gruber, M., *Wien. klin. Wchnschr.*, 1896, ix, 183, 204.

[2] Metchnikoff, E., Immunity in Infective Diseases, Cambridge, 1905.

[3] Metchnikoff, *loc. cit.*

in natural immunity against micro-organisms."[4] "The part played by agglutination in this immunity (acquired) is merely accidental and subordinate."[5]

After extensive investigation, Salimbeni[6] concluded that bacterial agglutination never takes place within the animal organism. This author worked with animals immunized to the cholera vibrio. The sera of the animals were strongly agglutinative *in vitro*, but he was unable to find any evidence indicative of agglutination *in vivo*.

Sawtschenko and Melkich[7] believed that agglutinins were present and active in the plasma of cases of recurrent fever. They found small clumps of spirochætes in blood immediately after its removal from the patients. It was suggested by them that the agglutination in the plasma was incomplete because of the rapid movement of the blood.

A glance at the literature given above readily convinces one of the slight consideration agglutinins as protective agents have received. Gruber's suggestions seem to have gone by as such and Sawtschenko's observations have not been considered sufficient proof of the functioning of agglutinins *in vivo*. Salimbeni has done the most direct and extensive work on this subject and his results led him to conclude that agglutination is strictly a phenomenon of the test-tube. And Metchnikoff is quite firmly convinced of the unimportance of agglutinins in both natural and acquired immunity. Our observations made in a more or less incidental manner seem to justify a different conception of agglutination as an immunity factor, for which reason they have been put together in this paper.

Pneumococci and Agglutination in Vivo.

In the course of the study of the manner of the rapid disappearance of pneumococci from the blood stream of rabbits following the injection of antipneumococcus serum the observation was made that an almost instantaneous agglutination of the pneumococci was produced in the blood by the serum introduced and, furthermore, that the clumped or massed cocci quickly accumulated in the internal organs.[8]

These facts in themselves are not only new, as is evident from the literature previously quoted, but they are important as bearing on our conception of the biological processes which come into play in the course of bacterial immunity. Aside from this consideration, however, the facts are of interest in connection with the following points: (1) the time required for the reaction to take place; (2) the

[4] Metchnikoff, *loc. cit.*, p. 258.
[5] Metchnikoff, *loc. cit.*, p. 263.
[6] Salimbeni, A. T., *Ann. de l'Inst. Pasteur*, 1897, xi, 277.
[7] Sawtschenko and Melkich, *Ann. de l'Inst. Pasteur*, 1901, xv, 497.
[8] Bull, C. G., *Jour. Exper. Med.*, 1915, xxii, 457.

degree of its specificity; (3) the concentration of serum necessary to effect agglutination; and (4) the relation of the quantity of serum employed to the course of a bacterial septicemia.

EXPERIMENTAL.

When the agglutination tests are made in test-tubes it is customary to incubate the tubes for two hours at 37° C. and then to set them aside at a low temperature for another period of several hours before the results are read.

In contradistinction to the slowness with which agglutination occurs and becomes evident *in vitro* is its instantaneous occurrence *in vivo*. To demonstrate this point we have proceeded as follows: The bacteria (pneumococci) from 50 cc. of bouillon are injected into the ear vein of a rabbit, after which a specimen of blood is taken from the heart to ascertain if sufficient bacteria are present to be easily found with the microscope and to determine the absence of clumps. The antiserum is then injected and a specimen of blood taken from the heart not later than thirty seconds afterwards. Other specimens are taken at one, two, three, five, and ten minute intervals. The sample of blood removed at twenty seconds usually contains the largest and greatest number of clumps. The second sample may also show many clumps of fair size, but the later samples show only a few small clumps. As a rule, no clumps or free bacteria whatever can be found at the expiration of five or ten minutes, particularly if from 1 to 2 cc. of serum have been injected.

Why bacteria agglutinate so much more rapidly *in vivo* than *in vitro* is not evident. The temperature conditions do not offer a satisfactory explanation, because the reaction does not occur in the test-tube immediately after body temperature is reached. The effect of the agitation caused by the circulation of the blood is not known, but would not seem to suffice to produce the great differences noted. It is quite possible that some constituent of the blood of the host aids the specific serum in producing those changes in the bacterial bodies which precede agglutination, but of this we are wholly ignorant.

The specificity of antipneumococcic agglutinins is as strict *in vivo* as *in vitro*. A heterologous serum causes no agglutination in a

concentration fifty times as great as that in which a homologous serum gives a positive reaction.

In following the reaction quantitatively *in vivo* we found that quantities of serum as small as 0.05 cc. per kilo of body weight of the rabbit caused the formation of small clumps, and that that quantity was the least amount that exercised any appreciable influence on the course of the septicemia or sufficed to prolong the life of the inoculated animals. Since the agglutinating titer of the antiserum employed was 1 to 50 by the macroscopic method, this result was wholly unexpected. But the result was rendered more comprehensive when it was ascertained that by merely modifying the method somewhat the titer could be increased to 1 to 500. Thus, when from 0.08 to 0.1 cc. of a bouillon culture of the pneumococci is added to 1 cc. of the diluted serum, clumps are formed which may be large enough to be seen by the naked eye, provided the fluids are clear. Stained preparations viewed with the microscope also reveal the clumps. But even so, agglutination within the body seems to take place with dilutions of the serum which are wholly ineffective in the test-tube.

Typhoid Bacilli and Agglutination in Vivo.

When typhoid bacilli are injected into the circulation of normal rabbits, they quickly leave the blood. The precise manner of the disappearance has not been investigated. Because the typhoid bacilli are subject to lysis it has been supposed that they are in fact disintegrated.

When, however, the blood was examined as described in connection with the pneumococci it was learned that the bacilli agglutinate within a few seconds after entering the blood stream. The rapidity with which agglutination takes place is affected by the power of the serum to cause agglutination in the test-tube. With rabbits whose blood does not possess this power a single bleeding could be performed before clumps were formed; in all others, agglutination had taken place within the brief period of thirty seconds.

The heating of typhoid bacilli to 80° C. for half an hour rendered them more readily agglutinable *in vivo* than unheated bacilli. While bacilli heated to from 65° to 80° C. proved inagglutinable in test-

tubes, our experience here confirmed the observation of others.[9] In this respect, therefore, agglutination *in vivo* differs from that *in vitro*.

Dysentery Bacilli and Agglutination in Vivo.

As a rule, normal rabbit serum does not agglutinate *in vitro* in dilutions of 1 to 10 any of the varieties of dysentery bacilli. The sera of all the rabbits we tested were inactive in this dilution. We employed the following strains: Shiga, Strong, Hiss, and Flexner.

The strains of the Shiga type do not agglutinate and remained in the blood for twenty minutes distributed uniformly; while the other strains undergo immediate agglutination, clumps being found twenty seconds after the bacteria were injected. By means of this reaction it was possible to determine within two minutes whether the culture tested belonged to the Shiga or Flexner groups of dysentery bacilli.

The fate of the dysentery bacilli could also be followed. When the rabbits are killed as early as seven minutes after receiving an inoculation of one of the Flexner group of bacilli, large numbers of leucocytes carrying clumps of one hundred or more bacilli were already present in the lungs, liver, and spleen.

When an immune serum for the Shiga type of bacillus is injected into the circulation the Shiga bacilli, which otherwise do not agglutinate, become immediately clumped. The distinction, therefore, in the behavior of the Shiga and the Flexner groups depends on the presence in the normal rabbits of agglutinins for the one and not for the other group.

Bacillus influenzæ and Agglutination in Vivo.

A non-virulent strain of *Bacillus influenzæ* isolated from the respiratory tract agglutinated in the circulation of normal rabbits one minute after injection. A virulent strain isolated from a case of influenzal meningitis was not agglutinated and was still in the circulating blood twenty minutes after injection. The distinction of virulent and non-virulent strains is determined by the fact that the former causes a fatal septicemia in young rabbits, while the latter does not.[10]

[9] Porges, O., *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 621.

[10] Wollstein, M., *Jour. Exper. Med.*, 1915, xxii, 445.

DISCUSSION.

The results described in the preceding pages emphasize the occurrence as well as the significance of agglutination of bacteria in the blood once they have gained access to the circulation.

In the first place, the power of the blood in normal animals to cause agglutination determines, apparently, in large measure whether the bacteria are to be promptly removed and septicemia avoided or to remain and to bring about without restraint that serious condition. The importance of this power as far as the normal rabbit is concerned is well illustrated by the examples afforded, on the one hand, by pneumococci, dysentery bacilli of the Shiga type, and virulent influenza bacilli, and, on the other hand, by typhoid bacilli, dysentery bacilli of the Flexner group, and non-virulent influenza bacilli.

The normal blood of the rabbit fails to agglutinate the Shiga dysentery bacilli outside (*in vitro*) or even inside the circulation, with the result that they are not converted into clumps in the blood stream and hence are not promptly removed from it, and while the normal blood does not agglutinate the Flexner group of dysentery bacilli in the test-tube in a 1 to 10 dilution even after two hours' incubation at 37° C., it does agglutinate them immediately in the circulation, whence they are quickly removed. Similarly, the circulating blood of the rabbit does not agglutinate pneumococci or the virulent form of influenza bacilli, with the result that they remain and multiply there, leading to a septicemia to which the animal succumbs; while typhoid bacilli and the non-virulent form of influenza bacilli are instantly agglutinated and promptly removed from the circulation.

In view of these facts we can discern in the phenomenon of agglutination in the blood an essential mechanism through which bacteria are so changed that they accumulate in the capillaries and sinuses of the viscera; and perceive in this mechanism the decisive act which determines whether protection is to be afforded or fatal septicemia supervene.

Indeed, the facts at hand illuminate the mechanism of protection from infection even further. It would appear that in the instances given the bacteria neither accumulate in the organs to any degree

when unagglutinated nor are they taken up by the leucocytes within the circulation when they remain there. Moreover, those bacteria which enter the organs before agglutination takes place remain in the single form and escape phagocytosis. When, however, agglutination has taken place the bacteria are quickly removed from the circulation and are englobed by leucocytes in the lungs, liver, spleen, and other organs, and, as may be inferred from the rapidity with which they are disintegrated in the phagocytes as compared with their persistence when free, are quickly destroyed, and possibly finally detoxicated. In other words, in order that the bacteria may be promptly removed from the blood stream it is requisite that they be first agglutinated, which condition is also required in order that they be destroyed *en masse* within the organs, a process achieved, apparently, chiefly through phagocytosis.

This phenomenon of protection in the normal animal is paralleled by what happens when an effective antiserum is employed to prevent or combat a bacterial infection, as is illustrated by the examples given of serum protection in pneumococcus and Shiga bacillus infection in the rabbit. It may be inferred that similar processes occur in other infections and in other animals, including man, but many more particular cases will need to be investigated before general deductions are made.

SUMMARY.

1. Small quantities of antiserum bring about instantaneous agglutination of pneumococci in the circulation of the rabbit; the reaction is specific and occurs in every case in which sufficient serum is given to influence the course of the septicemia or to prolong the life of the animal.

2. The agglutinating titer of antipneumococcus serum can be made considerably higher by adding only a small quantity of culture to the tests, thus making the test a finer differential.

3. Typhoid bacilli agglutinate spontaneously in the circulation of the normal rabbit; the reaction is positive *in vivo* even in cases in which undiluted serum gives a negative result *in vitro;* heating the bacilli to 80° C. for thirty minutes renders them more agglutinable *in vivo*.

4. Dysentery bacilli of the Shiga type do not agglutinate in the blood stream of the normal rabbit, but a small quantity of antiserum injected into the circulation causes immediate agglutination; while all strains of the Flexner group undergo spontaneous agglutination.

5. Non-virulent influenza bacilli agglutinate spontaneously in the circulation of the normal rabbit; virulent strains remain in the blood unclumped.

6. In all instances so far investigated of both passive and natural immunity, agglutination of the bacteria within the blood of the infected animal was followed by a rapid removal of the bacteria from the circulation, and by phagocytosis and destruction of the agglutinated bacteria in the capillary systems of the viscera; while those bacteria which are not agglutinated remain in the circulation and produce a progressive septicemia.

7. Hence the agglutinins seem to play the decisive part in at least certain instances of bacterial infections.

FATTY DEGENERATION OF THE CEREBRAL CORTEX IN THE PSYCHOSES, WITH SPECIAL REFERENCE TO DEMENTIA PRÆCOX.

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Plates 55 and 56.

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Object and Scope of the Investigation.

Although there exist in the literature many references to the normal and abnormal accumulation of fatty pigment or lipoid material in the nerve cells and neuroglia, it cannot be said that the subject has been treated exhaustively, or that our knowledge of the source and character and occurrence of this phenomenon is adequate. The significance of lipoid degeneration of nervous tissue and its relation, if any, to definite mental diseases has not been thoroughly investigated, and therefore it was thought important enough to make a thorough investigation of the subject and to ascertain if any possible relation existed between fatty degeneration of the nervous elements of the cortex and the various psychoses.

In order to determine this question, many difficulties arose, as the fatty content of the nervous elements in normal individuals had to be thoroughly studied before one could decide whether the accumulations of fatty pigment were pathognomonic or not. We know that these accumulations become more pronounced as the individual becomes older, so that it is important to consider the age of the individual before deciding upon the pathologic significance of this phenomenon. It is also possible that various poisons, especially alcohol, may have a potent effect upon the occurrence of fatty degeneration of the nervous elements, even in the adolescent and adult years of life. Therefore in order to establish any pathologic sig-

nificance to this phenomenon only a pronounced increase in the lipoid and fatty pigment out of proportion to the age of the patient can be regarded as patently pathologic. And this abnormal condition can be brought into definite relation to the morbid process only when it occurs regularly in many cases of the same disease. The object of this investigation then was to determine the relation of fatty deposits in the elements of the central nervous system as found in both normal and abnormal brains of human species. In order to determine whether it was a necessary constituent of nervous tissue, the brains of various wild and tame animals were also studied.

Methods.

The Herxheimer method[1] of staining the fatty pigment by means of Scharlach R was the principal method used, as this method is far superior to any other for demonstrating the fatty pigment, and it also enables us to make a comparative estimate of the character and amount of the pigment present.

For this purpose formalin-hardened material was used, and sections were cut with a freezing microtome. No alcohol material can be used, as the alcohol extracts a large amount of the fatty pigment, and the picture is therefore inaccurate.

Old formalin material can be used, although freshly hardened tissue is preferable.

[1] *Herxheimer's Stain for Fat by Scharlach R.*

1. Formalin fixation.
2. Frozen sections.
3*. Stain in Scharlach R solution 2 to 5 minutes, with or without heating. The stain is perhaps a trifle more intense with heat, but precipitation is more likely to occur.
4. Wash in water.
5. Stain in dilute Ehrlich's hematoxylin (diluted 1 to 4) for 1 to 3 minutes; usually a minute or a few seconds are sufficient if the solution is well ripened.
6. Wash well in tap water, 3 to 5 minutes or more.
7. Transfer to a slide by dipping the slide in the water beneath the section.
8. Dry off the water about the margins of the section with filter paper, but do not blot.
9. Mount in glycerine and seal the edges of the cover-glass with wax.

* *Scharlach Staining Solution.*

Make up just previous to use.

Saturate the following solution with Scharlach R in a test-tube by agitation and heat; filter carefully.

Absolute alcohol	70 cc.
10 per cent sodium hydrate solution	20 "
Distilled water	10 "

The Nissl stain was also used to determine other pathologic changes in the nervous elements. But frequently the picture obtained by the Nissl method gave no indication of the presence of the fatty pigment. Only in cases of far advanced degeneration such as that found in senile dementia was the fatty pigment demonstrable by this method.

The neurofibrils were studied by means of the Bielschowsky and Cajal methods, but the results obtained were not considered trustworthy on account of their difficulty and often unreliability. It was therefore impossible to form any opinion of the relations of the neurofibrils to the fatty degeneration, with the exception of a few types, notably the pathologic changes found in central neuritis and occasionally in senile dementia.

There can be no doubt that changes in the neurofibrils associated with fatty degeneration of the nerve cells exist, but with our present unreliable silver impregnation methods it is impossible to demonstrate this relation.

The Occurrence of Fatty Pigment in Brains of Normal Individuals.

This investigation was begun in the laboratory of the clinic at Munich in 1905–06. The difficulty of obtaining normal brain material in a community where the excessive use of alcohol was the rule soon became apparent. A number of normal brains were examined, but changes demonstrated by the Nissl as well as by the Scharlach method forced us to discard such material. In the interval (1906–11) normal material was collected principally from accident cases which came under the supervision of the county physician and from electrocuted cases at the State Prison. In this way we were able to examine the brains of about thirty cases of supposedly normal individuals of various ages. Many were not available because of excessive alcoholism, but we were able to form some opinion as to the occurrence of fatty pigment in various decades from the new-born to old age.

In a new-born no lipoid pigment was found in the glia, the ganglion cells, or in the cerebral cortex. In the case of a young man 16 years old, killed by an electric shock, lipoid granules were found in many ganglion cells of the cerebral cortex, but always in very small quantity only; they lay in the pyramid cells mostly at the base of the cells by the side of the nucleus, and produced the impression that they did not permeate the entire cell plasma but included only a comparatively small part of the cells. In the glia cells as well as in the vessels were found no single isolated fatty granules, and none were found in the superficial glia.

A considerable increase of the lipoid pigment was found in the case of an electrocuted convict 30 years old; here appeared likewise only very few fatty granules in the ganglion cells; usually only 3 to 10 granules lay at the base of the

cells near the border. As in the former case, so here in the glia and even in the glia cells of the most superficial strata no deposit of lipoid pigment was found. Neither were lipoid granules found in the ganglion cells of a 27 year old negro, electrocuted. In the case of a 36 year old suicide (because of unhappy family relations) somewhat more fatty deposit was found, still the amount was not very considerable. In a 70 year old man who had shown no mental defect the mass of the lipoid pigment in the ganglion cells was noticeably augmented. In many ganglion cells the entire base of the cells seemed to be filled with lipoid materials; the nucleus was also somewhat displaced toward the apical dendrite; and in the glia cells a large quantity of fat, likewise in the vessels, was demonstrated. Other brains of individuals, appearing mentally normal, were not available because during life a considerable abuse of alcoholic beverages had occurred, and a multiplication of the lipoid pigment in certain of these cases might probably have been connected with chronic alcoholism.

The result of these investigations may be summarized as follows: that lipoid materials are not to be found in the glia cells, the ganglion cells, and the vascular walls of the cerebral cortex of the new-born; that in the second decade of life lipoid materials already occur normally in the ganglion cells of the cerebral cortex, yet still in very small amount and only at particular points of the ganglion cells; that in the third period or decade they do not yet seem to be materially increased, but thereafter in later life the quantity assumes a considerable augmentation. While during the middle period of life only little fat is found in the glia cells and in the cells of the vascular walls together with a considerable increase in the ganglion cells, in advanced age the fat is increased in the glia and in the vessels; even in the most superficial strata of the glia there was found in middle age no accumulation of the lipoid materials. In the large Betz cells of the motor area, however, there is usually present a large amount of pigment even in normal individuals of various ages. Also in the cells of the anterior horn of the spinal cord fatty pigment is usually found in fairly large amount.

Hence we must conclude that in the large motor cells a considerable amount of fatty pigment is normal. It does not occur in the axis cylinder or even in the neighborhood of the base, but is usually found to one side of the nucleus and apparently does not interfere with the functioning of the cell.

The Occurrence of the Lipoid Pigment in Mental Disorders.

We have investigated the following mental disorders:

Amentia	1 case.
Intoxications { veronal poisoning, phosphorus poisoning	2 cases.
Chronic alcoholism	
Manic depressive insanity	10 "
Dementia præcox	20 "
General paralysis	12 "
Senile dementia	11 "
Anxiety psychosis	1 case.
Central neuritis	1 "
Circulatory psychosis	1 "
Idiocy	4 cases.
Epilepsy	19 "
Alcoholic insanity	10 "

In a case of infectious psychosis no augmentation of the lipoid substance in the cerebral cortex could be demonstrated out of proportion to the amount corresponding to the age of the patient. The same was true of the lipoid materials in the case of one dead of acute veronal and acute phosphorus poisoning. In individuals, victims of chronic alcoholism, an increase of the lipoid materials in all the elements of the cerebral cortex correspondent to the age of the patient appeared quite regularly. In the psychotic condition of chronic alcoholism, especially in the condition of dementia, the increase appears peculiarly rich, still none appears for the individual disorders of characteristic peculiarity.

In manic depressive insanity of the young there appeared no particular diminution of the amount nor change in the position of the lipoid materials compared with the normal. On the other hand, in the cases of later years a considerable amount of the lipoid materials was without doubt demonstrable, correspondent to the age of the person. Thus some cases which had repeatedly exhibited acute manic depressive conditions showed already at fifty years of age as rich a quantity of lipoid materials in the cerebral cortex as otherwise are usually to be seen only after sixty years of life.

Dementia Præcox.

Twenty cases of dementia præcox were examined. In this disease the estimation of the fatty degeneration was very difficult, because most of the cases ended fatally through exhaustion, especially from tuberculosis. Yet still there occur some cases among these in which death occurred very early or resulted from diseases which could hardly have exerted any essential influence on the cortical cells. We may therefore regard the pathological findings as a characteristic disease-process, in addition to the fact that these cases show remarkable similarity in the amount and distribution of the pigment. Because of the significance of the fatty pigment found in this group several cases are given in detail, with short abstracts. Cases dying of various intercurrent diseases are given to show the similarity of the cortical findings.

Case 1.—M. C. Female, 30 years old; married. Psychosis appeared after pregnancy, lasted four years. Paranoid type. Moderate dementia. Cause of death tuberculosis of the lungs. Section 15½ hours after death. No macroscopic alterations to be seen.

With the Nissl preparation there is seen a remarkable alteration of the cerebral cortex. Many cells are in a sclerotic state; in the superficial strata the glia are proliferated, and change in the architecture of the cerebral cortex is demonstrable. Several glia cells have penetrated many of the ganglion cells; the cellular body is slightly stained—almost invisible—and no more Nissl bodies are to be seen. With Scharlach preparations one sees a considerable increase of the lipoid substance. In the central lobe the small and medium pyramid cells are not so noticeably altered, but the Betz cells are strongly affected. The fatty pigment is dispersed in the entire cell body and is in great accumulation. Many cells are completely degenerated, and the cell is indicated only by the accumulation of the fatty pigment. In the superficial strata and also in the inferior strata the glia is largely fat. In the frontal lobes one sees almost all the small pyramid cells nearly wholly fat, and affected by this peculiar degeneration that is described later. The fatty pigment is deposited in the entire cellular body, especially at the base, and in many cells has penetrated the axis cylinder (Fig. 1). Frequently the nucleus is laterally displaced. The vessels contain much fat in the adventitia cells and in many small vessels the glia cells are found clumped about the vessel in which much fat is contained (Fig. 7).

Case 2.—A. D. Female, 24 years old; single. Duration of the disease, 1½ years. Teacher. Katatonic type. Refusal of food. Sense deceptions, delusions. Poison in food, etc. Moderate dementia. Died after 8 months in institution. Wasted. Cause of death tuberculosis of the lungs. Section 14¾ hours after death. No significant results are macroscopically demonstrable. Brain negative. On microscopic examination, with Nissl preparations one sees in the superficial as well as in the other layers that the glia cells are full of fatty pig-

ment. Mostly one sees a considerable increase in the glia cells, large protoplasmatic body, and fatty pigments. Usually they lie on the vessels, in many places six or eight consecutively.

The ganglion cells show marked fatty degeneration; this alteration corresponds to the sclerotic type (darkly stained nucleus and narrow cell body). The protoplasmic prolongations can be followed out very distinctly and most of the cells in the third stratum are plainly sclerotic, very slightly stained, and only shadows. These walls are attacked by large glia cells; usually one sees three to six glia cells in the cellular body of the ganglion cells. With the Nissl method the vessels showed no very considerable alteration. With the Scharlach method one sees at once in all cortical elements pathological alterations similar to those described in the foregoing case.

Case 3.—A. B. T. Female, 35 years old; married. Duration of the disease 4 years. Katatonic type. Two years before death cellulitis of the arms and legs. Profound dementia. Died suddenly, after some days, of volvulus and chronic nephritis. Section 5½ hours after death. Slight pachymeningitis, moderate chronic septic meningitis, moderate brain atrophy, pronounced ependymal granulation of fourth ventricle. The pathological findings in this case were, of course, not so well defined as in the two cases described above; still they are in accord in that there is present a pronounced fatty degeneration of all the nervous elements, which normally does not correspond to this age.

Case 4.—J. Died very suddenly of peritonitis, at 34 years of age. This case shows a far-reaching accord with the first case. Irregular arrangement of the fat in isolated little heaps over the entire cell, or string-like arrangement along the border of the ganglion cell. In most glia nuclei are found only a few fatty granules. The fatty granules of the glia satellite cells are often so dispersed over the cell that the fatty granules of the ganglion cell itself cannot be differentiated. Here and there one meets with individual glia cells richly endowed with fatty granules. In the vessels the fatty degeneration is not so marked.

From the study of our cases we conclude that in dementia præcox a profound degree of fatty degeneration in all cells of the cerebral cortex is found. In the ganglion cells the augmentation is often as rich as in senile dementia. Nevertheless it is indisputable that in most preparations much less fat is found than in senile dementia. It probably results therefrom that the fat is not found lying together in such thick clots as in senile dementia. And in general in the Nissl preparation they appear far paler and less yellowish than in the others. The profoundly fatty degenerated ganglion cells in dementia præcox exhibit a honeycomb structure, and the comb walls which enclose the structure slightly stained yet still recognizable through the powerful refraction of the light are much thicker than in senile dementia, where they are formed only of rows of fine granules, and finally may wholly vanish. Also in fibrillar pictures which have

been stained by the Cajal or Bielschowsky method we see relations different from senile dementia. We were not able to represent in such cells any finding or any deviations whatever of the fibrils, as is so easily done in senile dementia. But we notice the absence of fibrillar alterations, either because they really are not present, or because on account of the imperfection of the method they cannot be represented.

As a characteristic of all these cases must be noted that in all the cells of the cerebral cortex the lipoid materials were considerably augmented. The increase affected the entire cerebral cortex, but appeared most distinct in the second and third layers. Particularly striking pictures are obtained from the third stratum of the frontal lobe, where are found many ganglion cells whose entire cellular body is filled with lipoid substance. A frequent occurrence was the lipoid pigment continued on into the axis cylinder prolongation at its point of origin to the point of medullation. Such considerably extensive pictures of a similar sort can elsewhere be obtained only in senile dementia. With comparative preparations which were fixed with alcohol and stained with basic aniline, it is shown that all these cells present an advanced stage of sclerotic alterations, so that here also the fatty degeneration was connected with the sclerosis, as very frequently happens in chronic morbid conditions. In these preparations it is shown that the prolongations of the cells are stained remarkably far, often narrowed, often serpentine (especially in dendrites), the cellular body somewhat shriveled, the exterior forms remarkably angular. The nucleus is stained dark, often drawn out to an extraordinary length, often triangular or pyramid-formed, fitted to the shriveled cellular body. Often, too, in the exterior pyramid strata may be noticed a certain arrangement of the cortical elements in that the cells exhibit their apical dendrites not vertical to the superior surface, but placed in various directions, and are advanced noticeably far out of the cells. Not always did the accumulation of the fatty materials in the ganglion cells correspond to the accumulations in the glia. Sometimes they were found in regions in which the cells were profoundly fatty with only regressive alterations of the glia nuclei without lipoid granules. In other places, however, the glia showed rich fatty pigmentation and the

same condition shows itself also in the cells of the vascular wall. In the inferior strata where fewer acutely altered ganglion cells were found, more lipoid materials could, for the most part, be determined in the glia cells. Also the toluidin blue preparation showed that the glia nuclei in the second and third cortical strata could be recognized as affected by very considerable retrogressive alterations. They were in part extraordinarily pycnotic. Now and then such nuclei were found in the middle of a sclerotic ganglion cell. The glia cells of the superficial layer showed mostly a considerable fatty deposit, and in vessels of the inferior strata was found a large quantity of fat.

From this investigation there can indeed be no doubt that in cases of dementia præcox a considerable degenerative alteration in the cerebral cortex occurs, which alterations appear with special distinctness in the second and third cortical strata, and which lead to an extensive fatty pigmented sclerosis of the cortical elements. It can indeed be asserted that in many cases the greater part of the second and third strata is in such a condition that no further functioning can be expected. We have not been able to institute thorough examinations of the entire cerebral cortex, but the preparations from our cases give us the impression that the alteration in the frontal lobe is essentially more far-reaching than in the central convolutions. In this respect it is to be mentioned that in Case 1, where the ganglion cells in the frontal lobes were greatly loaded with fat, almost no accumulation of fatty pigment was found in the ganglion cells of the second and third strata of the central lobe. Also very little fat is found in the glia in either the superficial or deeper strata. The Betz cells are irregularly altered. In many cells there is shown a considerable accumulation of the fatty pigment which is not particularly distinguished from the normal condition, but in other cells the pigment is deposited around the nucleus. This irregular distribution of the fatty pigmented degeneration of the cortical elements of the cerebral cortex in dementia præcox possibly differentiates this process from senile dementia, since in the latter the whole cerebral cortex is affected equally.

Other Psychoses.

In a case of so called "anxiety psychosis," a disease of the climacteric period leading quickly to death, no pronounced increase of the lipoid pigment was exhibited, only it was striking that the fatty granules were deposited in the ganglion cells not in a collected accumulation, but distributed over the entire cellular body.

Very remarkable and unusual was the appearance of lipoid material in the nerve cells in a case of central neuritis, a peculiar terminal disease which was first described by Adolf Meyer[2] and later by others.[3] We will summarize the case briefly.

The symptoms of central neuritis gradually developed upon an apparent depressed phase of manic depressive insanity. They were general physical weakness, diarrhea, inclination to fall on the floor, later involuntary twitchings of all muscles of the extremities, followed by atrophy, stiffness in muscles, and pain upon movement. The peculiar confusion delirium occurred in connection with the twitchings and became even more profound with the progress of the other symptoms; later, unable to speak or to swallow, with profound hallucinations of a terrible or fearful type, and occupation delirium. Autopsy was made three hours after death and showed nothing specific macroscopically. In the cerebral cortex of the central lobe one sees at once the peculiar alterations of the Betz ganglion cells, the so called "axonal reaction" of Nissl, and first described by Adolf Meyer as characteristic of this disease. These alterations are accompanied by a pronounced degeneration of the white substance, as is illustrated by the Marchi stain.

On viewing the preparations with low magnifying power we are at once struck by the immense accumulation of lipoid materials in the cerebral cortex. Especially the exterior and the interior strata of the large pyramid cells are presented in many sections as a red stripe. In other sections the fatty degeneration seemed to be not entirely of so high a degree. When we examine the individual elements it is shown for the most part that the whole basic part of the ganglion cells even up to the processes is filled full with fatty granules, which appear, however, in many cases as no longer sharply isolated, but seem clustered together. The nucleus is then frequently displaced toward the apical process, and frequently one

[2] Meyer, A., On Parenchymatous Systemic Degeneration Mainly in the Central Nervous System, *Brain*, 1901, xxiv, 47.

[3] Cotton, H. A., and Southard, E. E., A Case of Central Neuritis with Autopsy, *Am. Jour. Insan.*, 1909, lxv, 633.

notices that on the border of the cell body large fatty spheres seem to project. It is not possible to determine here with certainty, however, whether we are confronted with an extrusion of the lipoid materials out of the ganglion cells, or whether these fatty globules belong to the satellite cells of the ganglion cells. In individual cells it is even possible definitely to assert that these large fatty globules, which in part reach to the compass of small glia grains, are encamped on glia nuclei. In isolated cases similar large fatty globules are found also in glia cells which are not satellite cells.

In the Betz pyramids we see no such masses of fatty deposits, but on the other hand we receive the impression that in their exterior part they contain many fine fatty granules which like a cloak veil over the fat-free interior of the cellular body (Fig. 6).

In the Nissl picture these Betz pyramids show changes which remind us of that cellular alteration which is observed in a transverse section of the peripheral nerves, in their ganglion cells (axonal reaction).

The nucleus has been shoved out to the border of the ganglion cells, and the Nissl bodies have undergone a dissolution. Where the ganglion cells are undergoing so advanced a stage of the disintegration progress we find also rich masses of fatty globules in the adventitial cells of the vessels. Besides this particular alteration of the Betz pyramid cells the small cells are pronouncedly fatty, but the type of fatty degeneration differs materially from the process described in dementia præcox. This mixed character of the fatty pigment of central neuritis is shown for comparison with dementia præcox (Fig. 6).

A case of circulatory psychosis,[4] or, as termed by the author, cardiogenetic psychosis,[5] was also thoroughly studied, and because it presented certain pathological features we report it here.

The patient was a negro woman, aged 55 years, who showed no symptoms until 8 days before admission to the State Hospital at Trenton. The psychosis was characterized by profound depression, motor restlessness, and extreme apprehensiveness, especially at night; marked variability of moods was present;

[4] Jakob, A., Zur Symptomatologie, Pathogenese und pathologischen Anatomie der "Kreislaufspsychosen," *Jour. f. Psychol. u. Neurol.*, 1909, xiv, 209.

[5] Cotton, H. A., and Hammond, F. S., Cardio-Genetic Psychoses, Report of a Case with Autopsy, *Am. Jour. Insan.*, 1911, lxvii, 467.

and frequently she was profoundly delirious, and at times seemed in a condition of hypomania. She died three months after admission, the cause of death being myocardial degeneration and edema of the lungs, and subacute adherent pericarditis. Autopsy three hours after death showed no macroscopic alteration of the brain.

In a general review it is at first to be determined that no focal or macroscopic organic disorders were present, and no inflammatory processes in the cerebral cortex. Only the nervous elements were attacked by the process,—the large elements of the inferior cerebral cortex, especially the Betz pyramids, and the small and medium cells. The most important alterations are in the Betz cells, and the type of the axonal reaction, as has already been described in the above case of central neuritis.

The findings here accord completely with the above cases. An extraordinary deposit of fat was found in the cerebral cortex, in conditions which suggest central neuritis. Only in this case the phenomena were not so prominent, the individual fatty granules had not coalesced into great masses, but the fatty granules had remained isolated as we have observed in senile dementia and the usual forms of fatty degeneration.

Senile Dementia.

We have examined eleven cases of this psychosis. The lipoid materials are multiplied in most of the cells where normally they lie in the ganglion cells. We see, however, in very many cells the fatty materials finally filling out the whole cell body. It is frequently noticed thereby that the cells are widened out by reason of the fatty deposit at the base, and that the cell nucleus is displaced toward the apical process. Also occasionally fat is seen in the cellular prolongations, and not very seldom the axis cylinder prolongations are indicated through a series of fatty granules. The fatty granules, even in senile dementia, are comparatively large; the entire cell body may have been filled full of fatty granules and the prolongations become invisible. In the Nissl preparation the spindles and Nissl bodies are lacking in the region of the fatty granules; on the other hand one often sees in their arrangement dark granules forming meshes between which the fatty granules are deposited.

The fibril picture at the point of the fatty degeneration of the ganglion cells is altered in a wholly characteristic manner. One may see that at the place of the fatty degeneration the fibrils are lacking; the fibrils do not run their otherwise customary course, but they lie

much rather at the place of the fatty degeneration and run their course in the border much thicker than usual or normal. We see single fibrils enter in the fatty locality and there pass over into meshwork. This meshwork may indeed not answer to a meshwork of fibrils, but a scaffolding of a web-like ground substance in whose meshes the lipoid materials are deposited. The fatty degeneration seems to affect in like manner all ganglion cells of all strata of the cerebral cortex. Particularly in many small cells one can recognize only the ganglion cellular body and the surrounding fatty granules. In many senile cortices there is found no ganglion cell which does not contain a large amount of fatty granules. The glia cells show in like manner a rich accumulation of fatty granules. A peculiarly rich fatty accumulation is found in many glia satellite cells. The fatty granules here often lie so close and massive on the ganglion cells that it is difficult even to separate them from the fatty granules of the ganglion cell.

Frequently those belonging to the glia cell are larger than those belonging to the ganglion cell, and thus the differentiation is facilitated. Often we find that the fatty granules belonging to the glia nucleus are situated somewhat on the side of the nucleus; so that occasionally also we meet with fatty granules which apparently seem to be lying free. On closer study of the section it becomes probable that also these little heaps belong to the glia nuclei (Fig. 5).

In senile dementia we seldom find glia cells without fatty deposit; both the large light staining cells and the small dark cells contain fatty granules. In the former the fat often stretches far away in a long tail of granules, or lies arranged in various gradations; though mostly so that the nucleus itself for the most part lies free. In the small dark nuclei we find often only single fatty granules. Usually the glia cells which lie in the neighborhood of the vessels are particularly rich in fat. In the vessels themselves there is found an extraordinarily large amount of fatty granules. They lie in large part in the adventitial cells; also in single intimal cells the whole cell is filled with fatty granules. In the adventitial space are found fatty granules which do not lie in the cellular body.

Progressive Paralysis.

Also in paralysis there is found a large amount of fat in the cerebral cortex, yet not so much as in senile dementia. Here and there we find cells in which the fat fills up the entire cell as in senile dementia. In general, however, a different arrangement is to be seen. The fatty granules seem to be much more strewn over the entire cell, while at no point do we reach such dense accumulations as are the rule in senile dementia (Fig. 1 a).

We have not seen fat in the prolongations of the cell, especially in the axis cylinder prolongations. Also a complete degeneration of the entire cell, as is frequent with senile dementia, seldom occurs. In our cases we have to deal with instances of paralysis predominantly running their course rapidly yet not wholly demented.

Also in glia cells fat is frequently found, but not in such an arrangement as is the rule in senile dementia. Occasional "*Stäbchen*" cells contain fat in the polar prolongations. In many cases the blood vessels contain large accumulations of fat, especially in the adventitial cells.

Epilepsy.

We have examined nineteen cases, among which were found various types of degeneration. We owe a part of our examined material to the Institution for Epileptics at Stettin, Würtemberg. For the most part the patients were epileptics in youth, and died at an early age. For the examination cases were selected in which no microscopic disease foci were found. Our material also included cases of status epilepticus from the Psychiatric Clinic at Munich.

Special interest is due to Case P., sixteen years old, who died in status epilepticus after he had suffered in earliest youth from teething convulsions. On the day before his death he had his first attack of status epilepticus, but previously had shown no epileptic symptoms. In the majority of the ganglion cells there is found a large quantity of fat in large grains which mostly lie in small groups at various points of the ganglion cells. In general the large cells are considerably more fatty than the small ones.

The glia cells of the first cortical stratum are richly endowed with fat. In the rest of the cortex the fat in the glia cells is very sparse.

Mostly one sees only single grains, especially in the satellite cellular body, while, on the other hand, the glia nuclei in the marrow contain a rich amount of fat. We see single cells which are surrounded with a large number of fine fatty granules. Here and there are found single adventitial cells surrounded with fat; most of the vessels exhibit less fatty deposit. In the lymph spaces of the large vessels of the marrow are found cells richly endowed with fat, but specifically fatty granular cells are not to be seen. Very rich is the fatty content of a second case of epilepsy which died in status epilepticus. We find here in all ganglion cells fatty granules remarkable by reason of their exceptional size. As a rule they lie singly, but dispersed over the entire cell, even far up to the polar prolongations. This was not observed in the other case.

In place of the small granules one sees small and large accumulations of fat, until a greater part of the cell is apparently filled with granules. The profound fatty degeneration of the ganglion cells corresponds also to considerable fatty deposit in the glia cells. A peculiarly beautiful appearance is made by the glia cells of the superficial layer which shows prolongations filled with very numerous fatty granules, stretching out from the cellular body in various directions. The abundance of glia cells in the first cortical stratum is noteworthy.

In the ganglion cells of the deeper layers and also in the glia cells we find fatty granules for the most part lying isolated. Their relation to the glia nucleus corresponds to the shriveled protoplasmic body. Very numerous are the fatty granules in the glia cells of the white substance where they often form rather coarse little heaps around the nucleus or are arranged in the form of a comet's tail springing away only in one direction. Finally the fatty content of the vessels is extraordinarily rich. All vessels are extremely prominent by reason of the large number of fatty granules deposited in them. Individual vessels are imbedded in the fatty granules. The lymph sheaths contain numerous cells with fine grains of deposited fat and also the adventitial as well as the intima cells seem to be completely filled with fat.

The fatty degeneration in the case of another young epileptic who presented an advanced grade of epileptic dementia and who died in

status epilepticus, while not in every way similar, is of a somewhat similar character. In individual ganglion cells the fatty degeneration reached a very high degree. Here too the fatty granules reach out far in all dendrites. There were found cells in which the dendrites seemed distended far from the nucleus through the deposit of fatty granules. Also here again our attention is struck by numerous cells in the first cortical stratum which send out dendrites in all directions richly endowed with fatty granules.

The glia cells of the whole cortex likewise show prolongations for some distance from the cell and filled with fatty granules. It often proved to be very difficult to differentiate the fatty granules belonging to the ganglion cells from those belonging to the glia cells. The glia cells of the white substance exhibit the same conditions as those in the preceding case. The vessels in general contain but little fat.

In still another case, an epileptic who died when eight years old of status epilepticus, the fatty deposits were of a peculiar nature. The ganglion cells here, through their form and the dark, often triangular nucleus, produce the impression of far advanced sclerosis. Many show very rich deposits stained red which, however, for the most part have not assumed the form of round granules. In general they are irregular, sometimes handle-formed, and often one end lies in the cell while the other projects out of it. In individual cells there occur also peculiar sausage-shaped formations. In many cases these reached a considerable length; they lie usually at the base of the cell and at the side of the nucleus. In between the usual fatty granules are often found. In general the fatty degeneration reaches a very advanced degree. Here again we see in many cells single fatty granules in the polar prolongations far from the cell nucleus.

In the glia cells of the deeper layers of the cortex are found very often fine fatty granules. Also in the glia cells of the white substance the fatty granules show an arrangement similar to that in the cases of epilepsy already described.

In the adventitial cells of the cortex and the marrow there are for the most part extremely coarse fatty granules in the vessels. In the adventitial lymph sheaths of the vessels there are rich and numerous granular cells.

Cerebral Syphilis.

In the two cases of cerebral syphilis that we have studied, one was a case of meningitis with peculiar participation of the spinal cord and the base, while over the hemispheres was to be demonstrated only a high grade thickening of the pia, with but very few infiltration cells. We were able to demonstrate also in the adventitial sheaths of the vessels of the superficial cortical stratum sparse lymphocytes lying wholly isolated. Before his death the patient presented the picture of a profound dementia. In almost all ganglion cells of the cerebral cortex there is a rich amount of fat. In most of the cells it lies at the base, so that the nucleus appears somewhat displaced upward, frequently mounts upward by the side of the nucleus, and still further occupies the region above the nucleus. The arrangement is very similar to that found in senile dementia. The fatty granules are large and give one the impression that the fatty accumulation pervades the protoplasm of the cell in its total depth. Around the glia nuclei lies abundant fat in very irregular form, and then again it is found deposited in single tails. Also in the glia of the white substance fat is found in like manner. The vessels are in part without fatty deposit. In other vessels fat is found in the adventitial cells, though not in excessive quantity.

The second case of cerebral syphilis is that of a woman in whom in the basal ganglia small and large focal softenings are found. In the hemispheres are shown no infiltration whatever and no alterations of the vessels. A long time before her death the patient presented the picture of profound dementia. The cortex here is shown to be extraordinarily rich in fat. The ganglion cells are for the most part full of coarse fatty granules, so that only a little of the remaining cellular body is to be seen.

The fatty granules appear to penetrate the entire cell and the whole cellular body. The large and small ganglion cells show the same degree of fatty degeneration. The picture of the glia is here peculiar. Around the small dark glia nuclei lie frequently little heaps of coarse fatty granules equally distributed in all directions. As a rule, elsewhere one does not see such an arrangement of the fat, since the fatty granules are mostly situated at the side of the nucleus, or lie arranged in some prolongations. In contrast with the extra-

ordinarily rich fatty deposits in the nerve and glia cells there is found but little fat in the vessels.

Alcoholism and Alcoholic Psychoses.

In M., a case of chronic alcoholism, the fatty degeneration of the ganglion cells is rather irregular. We find in all regions cells which contain but very little fat, and the small ganglion cells contain quite a large amount. In the large pyramids the fat lies at the base or at the side of the nucleus. Now and then we meet with a cell which contains a great quantity of fat. On the other hand, there is found an exceptionally high grade of fatty deposit in the glia. In all strata of the cerebral cortex are seen glia cells which are surrounded by coarse fatty granules. Sometimes the fat lies round about the nucleus; then again it is arranged in grape-cluster form at one side of the nucleus; and again it lies together in one or several tails running far on.

Not infrequently in several of them these fat-besieged cells lie side by side, so that large clusters of fatty granules lie together. Through the extraordinary accumulation of fat in the glia of the cerebral cortex the picture receives a wholly peculiar imprint. In the vessels there is quite a large amount of fat in the adventitial cells. *"Körnchen"* cells are nowhere to be seen.

In G. G., a case of alcoholic hallucinosis, aged 35 years, who died of dysentery, no fat was found in the ganglion cells of the frontal lobe or in the glia. In the temporal lobes, however, a moderate amount of fat was found in the ganglion cells, but none in the glia or adventitial cells of the blood vessels. It is important to note that no fat was found in the glia of the superficial layers of the cortex in any region.

In the other cases of chronic alcoholism and alcoholic dementia, varying in age from forty-eight to sixty-seven years, usually there was found a considerable amount of fatty pigment in ganglion cells, glia, and blood vessels, also in the superficial glia which resembled the fatty degeneration found in senile dementia, except that it was not of such a high grade. In no case was the peculiar fatty degeneration of the axis cylinders of the ganglion cell which

we have described in dementia præcox to be found. In the cases below sixty years of age the fatty deposits were greater than those found in normal brains of that age. So we must conclude that alcohol has a marked influence in the amount of fatty deposits in the cortex, when its use extends over a long period in the individual's life.

The Occurrence of the Lipoid Substance in the Cerebral Cortex of Animals.

We have had the opportunity to examine many examples of the cerebral cortex of various animals. In rabbits fat was not present in the cerebral cortex in any noteworthy amount. Just as little was to be found in an ape, although it had died of a general anemia on account of long confinement. On the other hand, a slight amount was found in the ganglion cells and the glia of a calf. A somewhat greater amount was found in the ganglion cells and in the glia of a full grown cow. But fat was found in the cerebral cortex of a deer which we had used for purposes of comparison in order to examine both domestic animals and those living in the open. The age of the deer could not be determined.

It is very unusual to find a large amount of fat in the ganglion cells and in the glia cells, and even when small amounts are found in ganglion cells and glia no fat is found in the adventitial cells. The results with a swine and a sheep were similar to those with the brain of the calf. In both of these cases the animals were young. In all these animals the deposit of fatty granules was collected at the base of the cells and was of small extent. When the fatty granules lay in the glia, as a rule only one or two were found in the cells. It could not be determined which cortical layers were rich or poor in fat. A much greater amount of fat was found in the brain of a horse which had reached the age of twenty-six years. Especially in the vessels there was a large amount of fatty pigment; it lay in the adventitial cells, and around the vessels were many glia cells containing a large amount of fat.

DISCUSSION.

The most important results of this study seem to be the demonstration of definite changes in the cortex in dementia præcox, which have hitherto escaped notice and have not been previously described.

Kraepelin[6] gives a reproduction of nerve cells from dementia præcox, with a high grade of fatty degeneration, but does not give credit to anyone in particular for these findings, although he evidently considers them of importance. The sclerotic changes in the nerve cells, as seen by the Nissl method, are also evidence of a profound pathologic change which cannot be ascribed to the age of the patient, for these cases are all from the early years, the third to the fourth decade.

We have been able to demonstrate these cell changes in practically all cases of dementia præcox, especially in those dying of an intercurrent disease; notably, tuberculosis. But in the case of patients affected with other psychoses, dying of tuberculosis, notably general paralysis, these changes are not to be found. It would therefore seem that tuberculosis alone could not account for these changes. What then is the significance of these changes? And the answer to that question cannot, as yet, be fully determined, although many interesting hypotheses could be discussed.

We cannot assume that the fatty degeneration or fatty deposits in the nerve cells, glia, and blood vessels are pathognomonic for dementia præcox, as we find similar changes in other psychoses, such as alcoholic insanity and senile dementia. But in no other psychosis do we find these changes at such an early age in the patient and never so pronounced. The type shown (Figs. 1 and 2) with the axis cylinder at its point of origin filled with fat appears to be found more commonly in dementia præcox. Occasionally one finds an indication of such a change in senile dementia (Fig. 8), but it is not so pronounced. Whole cells completely degenerated are found in both conditions. The fatty deposits in the superficial glia are about the same in both conditions, and are also found in alcoholic cases in advanced years of life. But the degeneration of

[6]Kraepelin, E., Psychiatrie. Ein Lehrbuch für Studierende und Ärzte, 8th edition, Leipzig, 1913, iii, pt. 2, 903, figure 191.

the axis cylinder and the fatty deposits in the superficial glia have not been observed in normal brains up to the sixth decade of life.

It is fair then to assume that these changes while not pathognomonic of dementia præcox are at least characteristic changes for that disease. The next question that arises is naturally: What is the cause of this fatty deposit in the cortex? And here the biochemical metabolism seems to play a very important part. With a better understanding of the function of the neuroglia and its relation to the nerve cells, this question does not seem so difficult. The classical work of Alzheimer upon the function of the glia gives us a better idea of just how the glia is concerned in the nourishment of the nerve cells, as well as its function in the removal of the products of metabolism in nervous tissue. It is fair to assume that the fatty deposits occur as a product of faulty metabolism, and also that it may be the end product of the activity of the cell, which under normal conditions is removed or neutralized by certain biochemical agents.

When this mechanism is at fault these end products are not disposed of, and hence accumulate in the cell bodies, glia, or walls of the blood vessels. The fact that the glia cells forming the superficial layer are loaded with fatty deposits would seem to substantiate this view. For the glia here usually show a marked reaction to any changes in the cortex, and it is possible to suppose that in the attempt to dispose of this product of metabolism they become the bearers of the fat, carrying it to the surface where an outlet can be obtained.

Without more experimental data one cannot hazard an opinion as to the nature and source of this product.

The findings of these changes, especially in dementia præcox, it is to be hoped will at least furnish the basis for further work in the field of metabolism, particularly that concerned with the glands of internal secretion.

In establishing these histopathological changes in dementia præcox, we believe that a foundation has been laid for considering dementia præcox an organic brain disease, and it is to be hoped that this work will stimulate investigation into the origin and nature of the lipoid pigment found in the cortex in this disease. The rela-

tion of the glands of internal secretion to the pathological process of dementia præcox offers the most fruitful field for investigation at the present time.

In conclusion I wish to express my sincere thanks and deep appreciation to Professor Alois Alzheimer for his valuable assistance to me in the laboratory of the Psychiatric Clinic in Munich. It was at his suggestion that this work was undertaken, and the greater part of it was done under his helpful direction. Without his cooperation and assistance this paper would not have been completed. I am also indebted to Dr. F. G. Scammell, County Physician at Trenton, for furnishing me with a greater part of the normal material, through accident and criminal cases coming under his jurisdiction; and to Dr. F. S. Hammond, Pathologist of the New Jersey State Hospital at Trenton, for his valuable assistance in the preparation of material, and in preparing the colored lumière plates from which the illustrations are made. The drawings were made by Dr. Peter F. Mallon from the colored photomicrograph.

CONCLUSIONS.

From the data provided by our investigations we may conclude that in all pathologic processes of the cortex which end in dementia and death, the fatty degeneration of the elements of the cortex plays a not unimportant part. The characteristic change for most of the psychoses is found in a great increase in amount of fatty deposits when compared to normal individuals of the same age. In some processes such as senile dementia and dementia præcox the fatty substance appears to fill completely the cell body, and these cells have apparently lost all their functioning power. It is not common to find the fatty deposits in the processes of the ganglion cells except in dementia præcox, and to a limited extent in senile dementia.

In other cases the pathological variety of the fatty deposits in the ganglion cells is seen to be diffused over the whole ganglion cell. We were able especially to observe this in infectious psychoses, in general paralysis, and in epilepsy. The so called central neuritis assumes a peculiar attitude in that it plainly leads swiftly to an acute fatty degeneration of the ganglion cells, in which there exists

an inclination of the fatty granules to flow together into large masses. Frequently the fatty degeneration of the ganglion cells appears to be connected with the sclerosis of the cells, especially when it is a matter of slowly progressing alterations of degeneration. The behavior of the glia is not wholly uniform in the various disease processes. In chronic disease processes we often find that the extent of the fatty accumulations in the ganglion cells does not correspond to an equal increase in the glia cells, while the otherwise acutely degenerative alteration in the nuclei of the glia is noticeable. In acute processes we see regularly an equal accumulation of the fat in both species of cell. The conditions of the cells in the vascular wall are wholly similar to those of the glia cells. We must therefore assume that in chronic diseases the fatty substance has been carried out of the glia and the vascular walls while it has been retained longer in the ganglion cells. Among all the disease processes amaurotic idiocy assumes a peculiar position. We have observed that in addition to the fatty materials of the scarlet fat stain, still other fatty materials, lipoid in character, have made their appearance. While the study of the fatty deposit in the cerebral cortex offers some points for a differential diagnosis, yet it is not adopted in all cases, since the distinction in individual disease processes is not always characteristic. From the preceding examination, however, in many cases there result important findings which briefly we summarize as follows:

1. In all degenerative alterations in the cerebral cortex the mass of the lipoid materials in the ganglion cells in comparison with that in healthy individuals of equal age is found to be considerably augmented. In the alteration of the lipoid materials in the ganglion cells two types in general may be distinguished: (a) An augmentation of the lipoid materials in the ganglion cells, in places where normally a small amount of fat is found. (b) An augmentation of the lipoid materials over the entire cell.

2. The first type we find also characteristic in senile dementia. The second type occurs in acute infectious psychoses, general paralysis, and well advanced epilepsy.

3. While the advanced lipoid degeneration of the ganglion cells in senile dementia has already been described in many ways, it has

appeared from our investigations that also in the young chronic cases of dementia præcox far-reaching fatty degeneration of the ganglion cells, especially in the second and third cortical strata, likewise occurs. These findings should constitute an important contribution to the pathological anatomy of dementia præcox.

4. The so called central neuritis represents a peculiar disease process according to the appearance of the fatty degeneration, since this fatty degeneration reaches a very advanced degree, and also in so far as it deviates from other disease processes in that here there comes out very distinctly in the picture an inclination of the fatty granules to flow together.

5. Amaurotic idiocy also represents a particular disease process in respect to the lipoid degeneration, since here in addition to otherwise distributed scarlet stain lipoid materials, still other specific lipoid materials make their appearance.

EXPLANATION OF PLATES.

The drawings were made with an Eddinger drawing apparatus from colored photomicrographs of the original sections. The fatty material is stained red by Scharlach R and Ehrlich's hematoxylin is used as a counterstain for the cell body and nucleus.

ax. axis cylinder of cell.
nl. nucleolus.
gl. neuroglia.
g. ganglion cell.
adv. adventitial cells.

PLATE 55.

FIG. 1. Three ganglion cells from the 3d layer of the cerebral cortex of the frontal lobe, from a case of dementia præcox 27 years of age(all three of these cells were found in one fluid 1/12 oil immersion). a. Entire cell body filled with fat, only nucleus and nucleolus free. b. and c. Characteristic fatty deposit in base of cell and in axis cylinder which is much swollen and affected to the point of myelinization.

FIG. 2. A medium sized ganglion cell from third layer of cortex in another case of dementia præcox, 30 years of age, with fatty axis cylinder and base of cell filled with fat. In normal brains of this age practically no fat is found in cells of this type, shown in Figs. 1 and 2.

FIG. 3. Giant Betz cell from the motor cortex in the case of senile dementia, aged 69. A considerable amount of fat in the base of the cell. In normal brains these large motor cells contain some fat, but never as much as shown in this cell.

FIG. 4. Section from the superficial layer of the cortex, showing neuroglia cells filled with fat, from a case of dementia præcox, aged 30. Normal brains at this age show absolutely no fat in the superficial neuroglia. The amount of fat is only found in senile dementia.

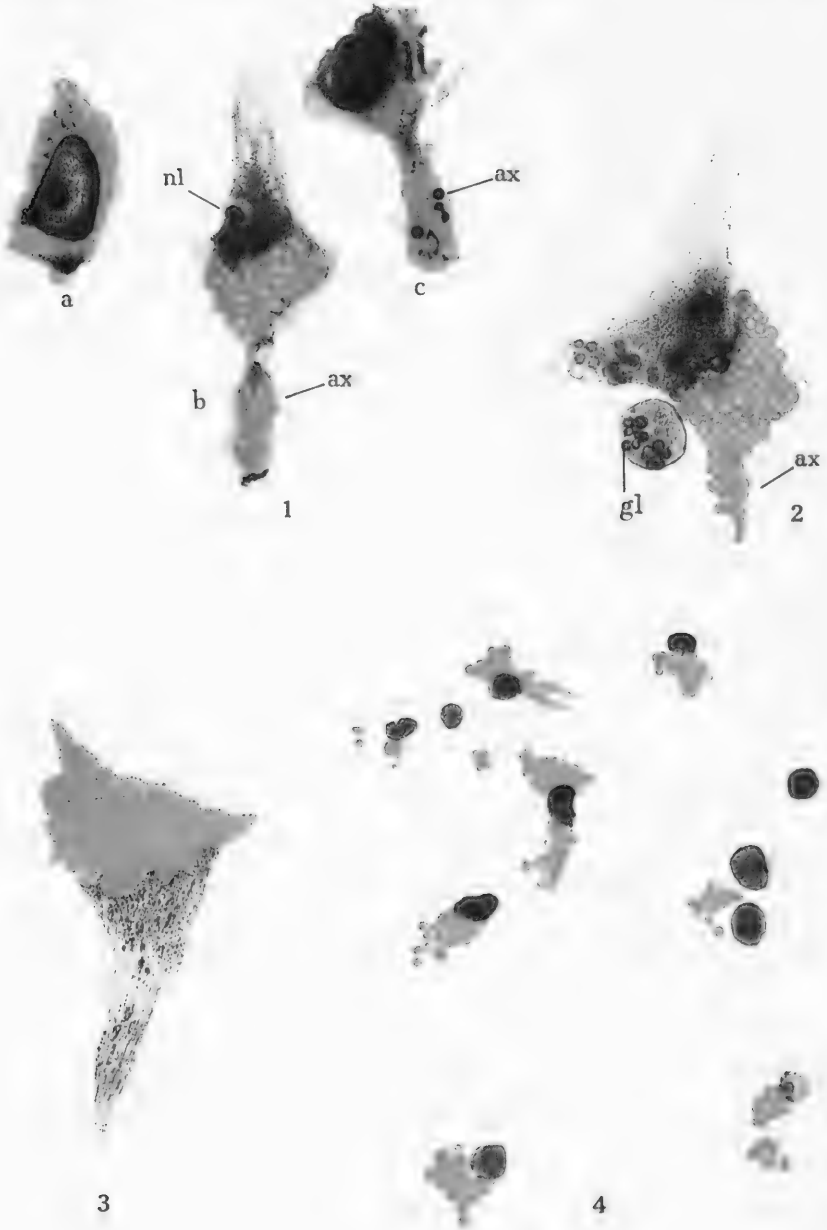
PLATE 56.

FIG. 5. A group of ganglion (g.) and neuroglia (gl.) cells from a case of senile dementia, showing the usual form of fatty degeneration in this disease. The cell body of the ganglion cell is filled with fat which surrounds the nucleus, but the axis cylinder is rarely affected. The glia cells contain more fat than is the case in dementia præcox (found in one field 1/12 oil immersion). The free fat outside of the cell probably belongs to the neuroglia cells.

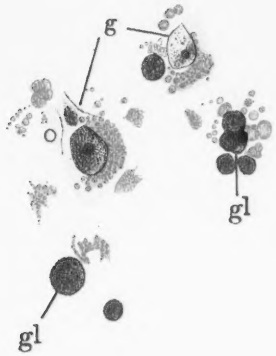
FIG. 6. Giant Betz cells from motor cortex from central neuritis. The nucleolus is eccentric and stains heavily; the center of the cell has lost its staining properties, surrounded by fatty material. Typical "axonal reaction."

FIG. 7. Blood vessel from the white substance of the brain from a case of dementia præcox, with considerable amount of fat in adventitial (adv.) cells. The glia cells lie close to the wall of the capillary and are filled with fat. These are not "*Körnchen*" cells, but ordinary glia cells, apparently transferring the fat to the blood vessels. This is a common finding in dementia præcox and is found only to great extent in senile dementia.

FIG. 8. Section of cortex from case of senile dementia, showing fatty degeneration of ganglion cells and glia. In one ganglion cell the axis cylinder (ax.) has some fat, but not to the extent as seen in dementia præcox. (Compare Fig. 1.)



(Cotton: Fatty Degeneration of Cerebral Cortex.)



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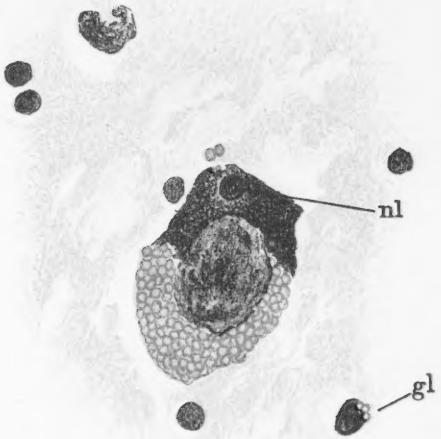

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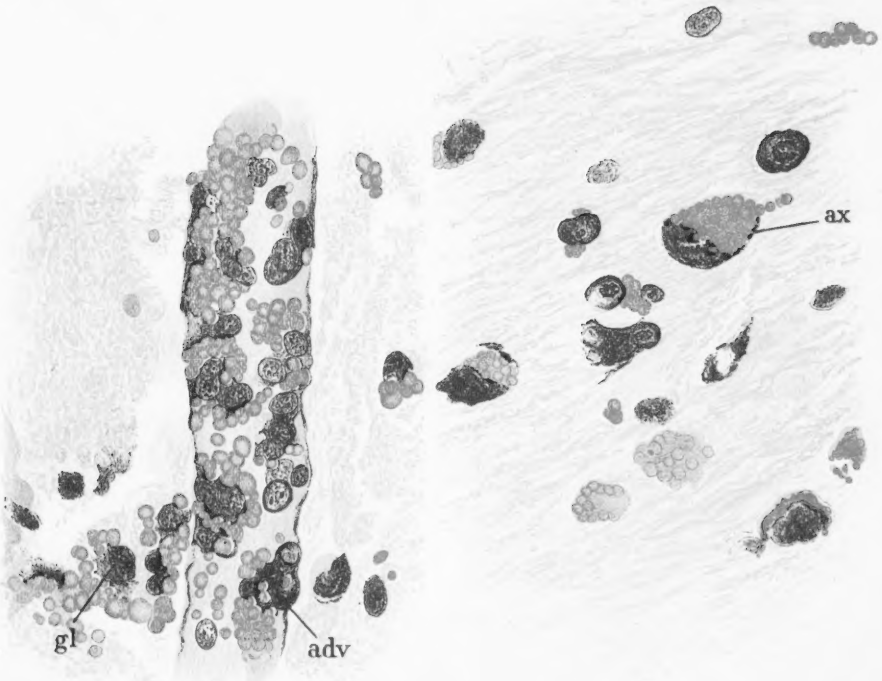

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(Cotton: Fatty Degeneration of Cerebral Cortex.)